STUDIES TOWARDS THE DIASTEREOSELECTIVE FORMATION OF THE MANUMYCIN *m*-C_7N CORE

by

R. Randy Spaetzel

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Name: R. Randy Spaetzel

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Examining Committee:

Chair: Dr. Robert Tait
Dean of Graduate Studies
University of Northern British Columbia

Supervisor: Dr. Guy Plourde, Associate Professor
Mathematical, Computer, and Physical Sciences Program
University of Northern British Columbia

Committee Member: Dr. Kerry Reimer, Associate Professor
Mathematical, Computer, and Physical Sciences Program
University of Northern British Columbia

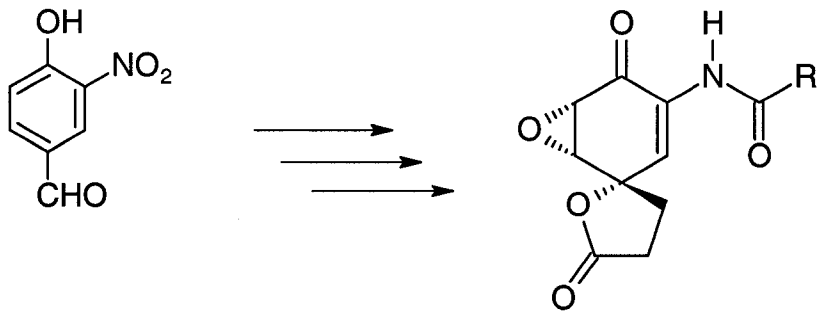
Committee Member: Dr. Waqar Haque, Associate Professor
Mathematical, Computer, and Physical Sciences Program
University of Northern British Columbia

External Examiner: Dr. Fraser Fleming, Associate Professor
Department of Chemistry and Biochemistry
Duquesne University (Pittsburgh, PA USA)

Date Approved: August 23rd, 2005

Abstract

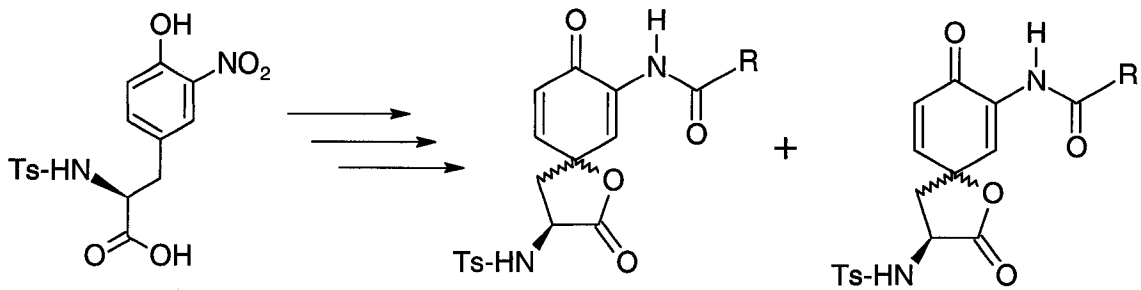
The thesis has three related parts, two modeling studies to investigate the chemical properties of oxidative spiroannulations and a third part using the information learned from the two modeling studies to induce facial diastereoselectivity for the formation of a *m*-C_7N unit analogue for manumycins. The 1st modeling study, involved the selective tosylation of the amino functional group on (L)-tyrosine and its subsequent spiroannulation. The 2nd part of the thesis, deals with an electron-donating functional group on various starting materials for the spiroannulations of racemic analogues of manumycins, (+/-)-**75/76,** and the effect the electron-donating group has on spiroannulations.



4-hydroxy-3-nitrobenzaldehyde (+/-)-75 / 76

The final part of the thesis entailed starting with a compound from the chiral pool, (L)-3-nitro-tyrosine, which we were able to use to synthesize compound (+)-**79** in five steps from the starting material, with a 3:1 ratio of

diastereoselectivity at the spirocarbon (total isolated yield of 85 %). Compound (+)-**79** represents the facial selective formation of a *para*-quinol analogue and is two steps away from being a *m*-C_7N unit analogue for manumycins.



67 (+) **-79** **80**

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Common Symbols and Abbreviations

μg	micrograms
μM	Micromolar
nM	nanomolar
AcCl	Acetyl Chloride
ATC	Anaplastic Thyroid Cancer
BF_3OEt_2	Boron trifluoride etherate
BOC	tert-butoxycarbonyl
BOC_2O	Di-tert-butyl dicarbonate
t-BuOOH	tert-butyl hydroperoxide
BzCl	Benzoyl Chloride
Cbz	Benzyloxycarbonyl
CF_3CO_2H	Trifluoroacetic acid
CH_2Cl_2	Dichloromethane
CH_3NO_2	Nitromethane
CO_2	Carbon dioxide
1-D	One dimensional
2-D	Two dimensional
DHEA	Dihydroepiandrosterone
DMSO	Dimethyl sulfoxide

ED_{50}	Effective Dose of 50%
EDG	Electron Donating Group
ee	Enantiomeric excess
Et_2O	Ether or Diethyl Ether
EtOAc	Ethyl Acetate
EtOH	Ethanol
EWG	Electron Withdrawing Group
H_2	Hydrogen
HBr	Hydrobromic Acid
HF	Hydrofluoric Acid
H_2O	Water
H_2O_2	Hydrogen Peroxide
HSP	Heat Shock Proteins
IC_{50}	Inhibitory Concentration of 50% Inhibition
In vitro	In the laboratory (outside the body)
In vivo	In the body
K_2CO_3	Potassium carbonate
KOH	Potassium hydroxide
KOTMS	Potassium trimethylsilanolate
$LiEt_3BH$	Lithium triethyl borohydride
$LiOBu^t$	Lithium tert-butoxide

LTA	Lead (IV) acetate
Me	Methyl
mg	Milligram
MeCN	Acetonitrile
MeOH	Methanol
MIC	Minimum Inhibitory Concentration
mL	Milliliters
$NaBH_4$	Sodium borohydride
NaH	Sodium hydride
NaOH	Sodium hydroxide
$Na[PhSeB(OEt)_3]$	Sodium phenylseleno(triethoxy)borate
Pd/C	Palladium on Carbon
PDC	Pyridinium Dichromate
Ph	Phenyl
PIDA	Phenyliodine diacetate
PIFA	Phenyliodine bistrifluoroacetate
ppm	Parts per million
PPTs	Pyridinium *p*-toluenesulfonate
PtO	Platinum oxide
Pyr	Pyridine
RP	Reverse phase

rt	Room temperature
TBDMS	Tetra-butyl dimethylsilyl
THF	Tetrahydrofuran
tlc	Thin layer chromatography
TMS	Tetramethylsilane
Ts	Toluene sulfonyl
TsCl	Toluene sulfonyl chloride
TsOH	*p*-Toluenesulfonic acid
TTN	Thallium (III) nitrate
VEGF	Vascular Endothelial Growth Factor

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Chapter 1

Introduction

(1.1) General

In nature, there is a conservation of oriented structural elements in three-dimensional organic compounds. A consequence of this conservation is that the amino acids and sugars which form proteins and carbohydrates, predominately favour one stereoisomer called an enantiomer. Enantiomers are molecules which are non-superimposable mirror images of each other, due to the molecules' structural elements oriented in a specific left or right handed order in 3-dimensional space around a central atom. The central atom of such a compound is said to be the chiral centre for the molecule and this chiral property of an enantiomer molecule is referred to as its chirality. Thus, these qualities of amino acids and sugars provide for the conservation of chirality throughout biological polymers that make up all living matter. As a consequence, metabolic or regulatory processes controlled by biological systems can be sensitive to a stereospecific enantiomer and this sensitivity is often a unique feature of enzymatic reactions and metabolic processes.[1] The degree of sensitivity to a pair of enantiomers is such that often there is a more active enantiomer (eutomer) for a given action of interest, while the other enantiomer (distomer) can have a wide range of varying actions. The possible range of actions for the distomer can include having: partial biological activity, no biological activity, different rates of

metabolism, or being antagonistic to the original eutomer's biological activity, or having a different biological activity all together, that is either desirable or undesirable.[2]

The paradigm shift caused by the advance in stereoselective bioanalysis and stereoselective technologies in the 1980s has led to this new awareness of the sensitivity of biological processes to enantiomers. Previously, racemic compounds (an even mixture of enantiomers) have dominated the pharmaceutical industry. However, when considering the possible different biological effects that eutomers and distomers have, it is clear that using enantiopure drugs has had major advantages. These advantages and disadvantages contributed to regulatory controls being established with the publication of formal guidelines in a document entitled *Policy Statement for the Development of New Stereoisomeric Drugs* in 1992 by the United States.[3] The European Union followed the United States' lead in 1994 with similar guidelines in the document *Investigation of Chiral Active Substances.*[4] Essentially, all applicants seeking drug approval must now recognize the occurrence of chirality in new drugs. Attempts must be made to separate the enantiomers and evaluate each stereoisomer's contribution to the perceived medicinal benefits of the substance. The benefits of the enantiomers are then examined individually or together as a racemic mixture in order to make a rational selection of the stereoisomeric form that is to be marketed.[2]

Worldwide sales of enantiopure drugs continue to increase at significant rates. For example, in 1996 there was a 27% (US $ 74.4 billion) increase of

enantiopure compounds marketed. This trend has continually increased each year to an impressive 39 % (US $151.9 billion) in 2002.[2]

Many of the new enantiopure pharmaceuticals are the result of research examining compounds isolated from natural products. The isolated natural compounds are subjected to a series of broad based screenings for biological activity to determine the compound's biological worth. As a continuation of this process organic chemists use three broad research streams in order to increase the biological activity of potential lead compounds: (1) using the natural product to build new analogs and derivatives, (2) the diversity-orientated synthetic route to new skeletons, and (3) creating new derivatives based upon paths made available by total synthesis of the natural product.[5]

The approach used in this thesis was based on the third research method, thus, creating new derivatives based upon paths made available by the total synthesis of the natural product. But instead of using total synthetic routes already established, the primary goal of the research presented here was to develop a novel stereoselective route to new natural product analogues of the manumycin family.

(1.2) Manumycins

The manumycin family of compounds are a class of secondary metabolites isolated from microbial origins that, presently, consist of 28 similarly structured molecules.[6, 7] The term for this class of compounds originated from Manumycin A **1**, (previously called Manumycin), the first member of this family of compounds

isolated by Zähner and co-workers in 1963.[6] In 1973, Schröder and Zeeck proposed a novel structure for Manumycin A **1** but the stereochemistry was later revised by Taylor and co-workers after they reported their synthesis of the enantiomer of the natural product.[8] The common structural elements used to classify manumycins are: two unsaturated carbon chains, attached meta-fashioned to a distinctive functionalised cyclic core (see Figure 1).[9]

The distinctive cyclic core of manumycins, often referred to as an *m*-C_7N unit, can

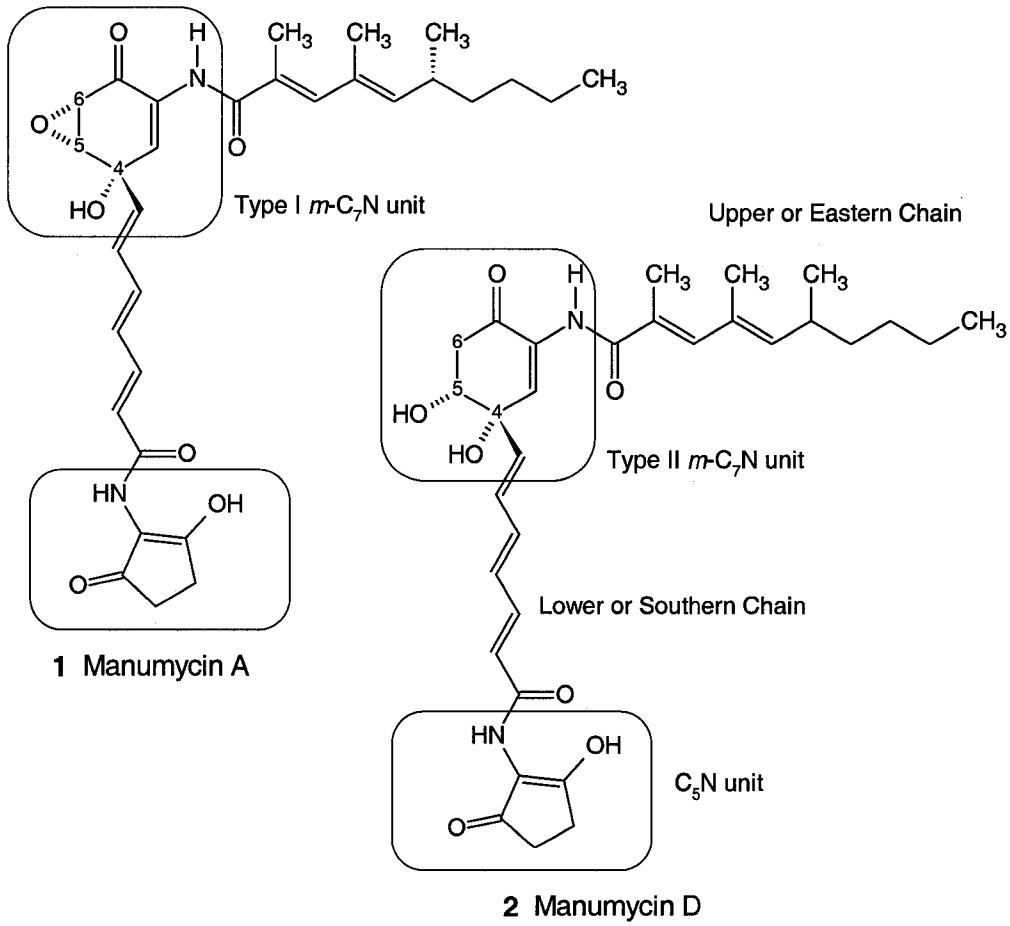


Figure 1 Structural elements of Manumycin A and Manumycin D [11]

exist as either a type I or a type II configuration. The type I configuration has an oxirane at the C-5/C-6 carbons of the cyclic core *m*-C_7N unit, while the type II configuration has a hydroxylethylene at the C-5/C-6 carbons of its *m*-C_7N unit, as illustrated in Figure 1 with Manumycin A **1** (type I) and Manumycin D **2** (type II) respectively.[10, 7] Another common feature to many of the manumycins is a 2-amino-3-hydroxycyclopent-2-enone moiety (C_5N unit) linked to the "lower" or "southern" chain as shown in compound **2**. While the family of manumycins retain many similar structural elements, the significant structural differences between manumycins occur mostly in the "upper" or "eastern" chain. These structural variations of the "upper" chain involve different patterns of methyl branches and double bonds with varying lengths of polyunsaturated carbon chains attached to the *m*-C_7N unit. [11] (For a complete table of Manumycins see Appendix 2).

(1.3) Origin of Manumycins

All manumycins come from micro-organisms isolated from soil samples collected worldwide.[11] The micro-organisms are taxonomically characterized as actinomycetes (genus: Streptomycetes), gram positive, mycelical, and sporulating bacteria. The specific properties which have characterized manumycins' discovery during natural product research are antibacterial and/or the inhibition of the two enzymes farnesyltransferase or interleukin-1β. Typically, the different types of bacteria producing manumycins are cultivated using the standard procedures for Streptomycetes strains. The manumycins can then be isolated from either the

culture filtrate or the mycelium. Isolation of the compound is accomplished with standard procedures involving organic solvent extraction techniques of the culture filtrate or the mycelium, followed by purification using column chromatography on silica gel or reverse phase (RP) silica gel. One other separation technique sometime utilized for the purification of manumycins is gel-permeation chromatography on Sephadex® LH-20.[12]

(1.4) Biosynthetic Pathways of Manumycins

Researchers have attempted to determine the biosynthetic pathways of manumycins using the first two manumycins discovered: Manumycin A **1** and Asukamycin[13] (see structure in Appendix 2). The technique used to determine the biosynthetic building blocks of the bacteria's pathways involves feeding studies using stable radioactive isotope-labelled precursors fed to the bacteria. [13-15] Currently, the formation order or mechanism of the building blocks that make the *m*-C_7N unit is unknown. [13] The *m*-C_7N unit is believed to come from combining a four carbon dicarboxylic acid derivative, like succinate or oxalacetate, with one molecule of a glycerol metabolite. While the epoxides and hydroxyl functional groups of the *m*-C_7N unit are known to come from molecular oxygen, the carbonyl functional group does not come from molecular oxygen. The nitrogen of the *m*-C_7N unit is believed to have come from somewhere within the nitrogen pool. But once the *m*-C_7N unit is formed, bacteria then initiates the assembly of the "lower" chain in both compounds using three malonyl-CoA substituents. The C_5N unit at

the end of the chain comes from an intramolecular cyclization of δ-aminolevulinic acid. The "upper" chains' cellular pathways appear to be assembled differently for Manumycin A **1** and Asukamycin; however, both come from a polyketide origin. These differences in the "upper" chain cellular pathways should not be surprising considering that this is where most structural differences in the manumycin secondary metabolites are found.[13]

(1.5) Biological Activity of Manumycins

The manumycins show many interesting biological properties *in vitro* and *in vivo* which so far include: antibiotic, antifungal, antiparasitic, anticoccidial, trypanocide, and insecticidal activites.[11] Manumycins' biological properties have led to a number of patents being issued for their various applications and possible economic potentials.

Another biological activity manumycins possess is the ability to inhibit enzymes in plants and animals. The inhibition of plants enzymes could possibly lead to treatments to help alleviate drought by Manumycin A's **1** ability to regulate the stomata openings in plants. In humans, the inhibition of certain enzymes could conceivably be used in potential cancer therapies.

(1.5.1) Antibacterial Activity of Manumycins

Manumycins with the structural type I *m*-C_7N configuration display antibacterial activity to gram positive bacteria. [16] However, manumycins with the

structural type II *m*-C_7N configuration do not exhibit any antibacterial properties. This difference could lead one to suggest that the epoxide on the *m*-C_7N unit is a necessary component of the manumycins antibacterial properties.[7] Some of the gram positive bacteria tested with various manumycins include: 11 different types of *Staphylococcus* with a minimum inhibitory concentration (**MIC**) range of 0.39-16 micrograms per millilitre ($\mu g\ mL^{-1}$), 2 different types of *Streptococcus faecalis* with a MIC of 6.4 ($\mu g\ mL^{-1}$), 3 different types of *Micrococcus* with a MIC range of 0.39-16 ($\mu g\ mL^{-1}$), and 9 different types of *Bacillus* with a MIC range of 0.19-12.5 ($\mu g\ mL^{-1}$).[16-19]

Most of the manumycins tested did not inhibit gram negative bacteria growth. Surprisingly, Manumycins E, F and G did inhibit a gram negative bacteria, demonstrating a MIC range of 0.1-2.5 ($\mu g\ mL^{-1}$) for *Escherichia coli* SGB888.[16-19] The biological manner in which manumycins inhibit both types of bacteria *in vitro* is not known and, to date, no drug resistant bacteria have been tested. However, due to their prohibitive costs, manumycins are not currently ideal candidates for antibiotic applications. As of May 2004, the cost of Manumycin A was: 1 milligram (**mg**) US $ 40.00, 5 mg US $ 120.00, and 10 mg US $ 190.00, from A.G. Scientific, Inc.[20] However, there have been a number of patents issued for various manumycins and their derivatives which have also included their antibiotic properties. Some examples of these patents are for the compounds Asukamycin [21], Alisamycin [22], Manumycin C, Manumycin D, Manumycin E [23], and for manumycin type epoxycyclohexenedione derivatives.[24,25]

(1.5.2) Antifungal Activity of Manumycins

The extensive use of fungicides worldwide is a serious environmental concern and has concurrently increased the number of resistant strains of fungi.[26] However, antibiotics derived from microbial origins generally have fewer environmental side effects and show little toxicity towards the host plant. The microbial antibiotics also have selective inhibitory activity and are able to decompose quickly, thus lessoning their environmental impact. Presently, there are manumycins and manumycin derivatives that have exhibited antifungal activity for a number of different fungi.[26] The fungus *Candida albicans* is inhibited by Manumycin A **1** (MIC 21 μg mL $^{-1}$) [9], Alisamycin (MIC 10 μg mL $^{-1}$) [19], two benzoquinone derivatives of Manumycin B (MIC 10 μg mL $^{-1}$) and Manumycin C (MIC 42 μg mL $^{-1}$).[27] Asukamycin was reported to have a weak antifungal activity against *Trichophyton mentagrophytes* at a moderate concentration (MIC 25 μg mL $^{-1}$).[18] Another manumycin derivative, SW-B (2,4,6,-trimethyldeca-(2E,4E)-dienamide), an amide composition of the upper chain of both Manumycin A **1** and Manumycin D **2**, showed a high level of inhibitory activity against *P. capsici, M. grisea, C. cucumerinum, and M. grisea.* [26] Hyphal growth of the pervious fungi were inhibited by more than 50% at a level of 10 μg mL $^{-1}$ and by about 90% at 50 μg mL $^{-1}$. Further research with manumycins may help produce new fungicides treatments that address the environmental concerns due to excessive applications of broad range fungicides.

(1.5.3) Antiparasitic Activity of Manumycins

Eimeria belongs to the phylum Apicomplexa and is closely related to the infamous parasites *Plasmodium*, *Toxoplasma* and *Cryptosporidium*. *Plasmodium* is the causative agent of the disease malaria, and *Toxoplasma* is an opportunistic parasite associated with AIDS and some congenital birth defects. *Cryptosporidium* is a worldwide waterborne parasite causing severe diarrhoea. *Eimeria* are intracellular protozoan parasites that can cause significant disease and death among cattle and poultry, thus inflicting severe economical hardship on those industries. Specifically, *Eimeria tenella* develops within epithelial cells that line the intestinal tract of domestic fowls and likely infects 30 billion chickens annually.[28] When Asukamycin was added to the feed (at 100 parts per million (**ppm**)) of 4 day old chickens infected with *Eimeria tenella*, it was found to inhibit coccidial activity in their intestines.[18]

Trypanosoma brucei, another parasite found mainly in Eastern and Southern Africa, is spread by the blood sucking Tsetse fly and is the cause of "sleeping sickness" in humans. Unfortunately, prospects for a vaccine against the parasite are low, and more worrisome, is the fact that the parasites are starting to develop resistance to the compounds presently used to combat them.[29] A number of farnesyltransferase inhibitors were tested for their efficacy against *Trypanosoma brucei*. Surprisingly, only Manumycin A **1** and a number of racemic quinone manumycin analogues were effective against the parasite.[30] The racemic

analogues being tested had the structural features of Manumycin A **1** but, surprisingly, the researcher did not explore if there were any possible differences in enantioselective efficacy. While Manumycin A **1** and the quinone analogues inhibited the growth of the parasites in both the vertebrate's bloodstream and the procyclic form within the Tsetse flies, it was not just the farnesyltransferase inhibiting action that affected the parasites. A post morphological analysis showed there was significant mitochondrial damage within the parasite. Further testing indicated that Manumycin A **1** also affects the mitochondria of mammalian cells in a similar way, but to a much lesser degree.[30] A patent (in January 2001) was issued for the properties of manumycin A and manumycin type analogues as an application to treat or prevent numerous parasitic disorders by the World Intellectual Property Organization (**WIPO**).[31]

(1.5.4) Insecticidal Activity of Manumycins

Manumycin A **1** was tested for its efficacy as an insecticide agent against *Lepidoptera* (butterflies and moths) and *Coleoptera* (beetles).[9] By applying an aqueous solution of Manumycin A **1** (0.05 %) to the vulnerable plants, Manumycin A **1** acted as an insecticidal development restrictor on the eggs and larvae of *Pieris brassicae* (cabbage white butterflies) and *Epilachna varivestis* (Mexican bean beetles). After the initial application, a repellent effect was also observed on larvae intending to feed on the same plants treated with Manumycin A **1**. Unfortunately, higher concentrations of Manumycin A **1** did not increase the

efficacy of the insecticide and was slower to work than a comparable chitin synthase inhibitor.

(1.5.5) Plant Enzyme Inhibition by Manumycins

Stomata are pores found on the outer layer of the aerial parts of most plants. The stomatal opening of the plant is used to regulate the gas exchange between the atmosphere and the interior of the plant. Therefore, the opening and closing of the stomata influences two important processes of vegetative plants: (1) photosynthesis with the uptake of CO_2 and (2) transpiration with the evaporation of water.[32] Plants lose over 90 % of their water through transpiration. The phyto-hormone abscisic acid prevents this water loss by closing the stomatal aperture via the guard cells. It has been discovered that this process can be regulated through the inhibition of farnesyltransferase using Manumycin A **1**. Thus, Manumycin A **1** or a Manumycin derivative could be utilized as a means to enable plants to better survive droughts.[33] Currently, a patent application is being reviewed by the WIPO, which proposes using the inhibition of farnesyltransferase as an application to control the effects of droughts on plants.[34]

(1.5.6) Inhibition of Enzymes by Manumycins

The first mammalian enzyme activity describing manumycins was the inhibition of the human polymorphonuclear elastase by Manumycin A **1** with an inhibitory concentration of 50 % inhibition ($\mathbf{IC_{50}}$) = 4.0 μg mL^{-1}.[35] The secretion

of elastase by tumour cells to destroy elastin (a protein responsible for the elasticity of body tissues) is important in the processes of tumour cell invasion and the metastasis of tumours.

In 1993, during yeast based screening assays, Manumycins A **1**, B, C and their benzoquinone analogues were discovered to be inhibitors of Ras farnesyltransferase.[27,36] Manumycins A **1**, B, C have an IC_{50} = 5, 13, 7 micromolar (μ**M**) respectively for the inhibition of Ras farnesyltransferase. Part of the study examined the benzoquinone analogues of manumycins A **1**, B and C that lack the "lower" side chain. It is important to note the lack of the "lower" side chain did not significantly reduce inhibitory activity of the benzoquinone analogues. The Ras farnesyltransferase enzyme is known to activate the Ras proteins by transferring a farnesyl residue from farnesyl diphosphate to a sulphur atom of the cysteine amino acid on the Ras protein. This is the first of four steps involved in the post-translational modification needed to activate many Ras proteins. Once the four modifications take place on the Ras proteins, the Ras protein attaches covalently to the inner cell membrane and act as relay signals from the cell surfaced receptors to the nucleus. It is through control of this cellular pathway that cell proliferation is stimulated and regulated.

The superfamily of Ras proteins are currently being studied because of their role in cell growth and their suspected association with 30 % of all cancers.[37] In mammalian cells there are three types of Ras proteins: H-Ras, K-Ras, and N-Ras proteins.[38] In at least 17 different types of cancer, mutated Ras proteins have been

isolated and identified. These mutant Ras proteins are suspected to be a factor in the malignant growth of solid tumours in 90% of pancreatic cancers, 50% of colon cancers, and 30% of lung cancers.[39]

Ras proteins were originally named after the *ras* gene, which was first identified in viruses that caused rat sarcoma.[40] Researchers have speculated that mutated Ras proteins are believed to come from ras oncogenes that have been incorporated into organisms by viruses. Once incorporated in the organism, they are thought to be conserved through evolution in organisms as divergent from humans to yeast.[40]

During further *in vitro* screenings, manumycins were found to inhibit caspase-1, also called interleukin-1β converting enzyme (**ICE**).[35, 41] Interleukins are produced by immune system cells such as lymphocytes, macrophages and monocytes. Interleukins are regulatory proteins that are members of the larger family of cytokines. Cytokines control inflammation and immunity by regulating both growth and differentiation of lymphoid and other cells. ICE has been suspected of being involved in acute and chronic inflammation. The Caspase family of protein enzymes are linked to the regulation of inflammation and programmed cell death (**apoptosis**). Caspase-1 and Caspase-11 regulate and control inflammation while the other 11 mammalian caspases are believed to initiate and control apoptosis. During the *in vitro* screening, eight manumycins and a few of their benzoquinone analogues exhibited inhibitory activity towards caspase-1, with the manumycin EI-1511-3 (see Appendix 2) demonstrating a

notable IC_{50} = 90 nanomolar (**nM**) of inhibitory activity.[35,41] Hence, manumycins or their analogues could become a prospective treatment for various inflammatory diseases.

Additional research has uncovered a number of other enzymes that are also inhibited by manumycins. Manumycins and their benzoquinone analogues are irreversible inhibitors of neutral sphingomyelinase.[42] Sphingolipids were long considered to be inert lipids of cell membranes, but have recently been shown to have roles in cell biology and signal transduction.[43] Sphingolipid ceramide is regulated by the sphingomyelinase enzyme and plays a pivotal role in the sphingomyelin cellular pathway.[43] Ceramide is a regulated product of this pathway and is thought to trigger and/or control proliferation, differentiation, growth arrest and apoptosis in cells. Recent studies strongly suggest that ceramide plays a vital role in inflammations and tumour suppression. Further research in the sphingomyelin cellular pathway may help produce new therapeutic treatments.[42]

(1.6) Investigation of Manumycins for Medicinal Properties

Recent advances in biochemistry and genetic research on the complexities of biological processes within the cell has afforded the development of new methods to combat previously incurable diseases.[44] However, the need for new therapeutic agents is evident from our limited success with the treatment of so many diseases. As our understanding of the intricate cellular pathways within the cell increases, we are able to manipulate a number of pathways through inhibitors

with increasing success. One of the cellular pathways currently under investigation is the use of Ras farnesyltransferase inhibitors as potential therapeutic agents.[44]

(1.6.1) Inhibition of Corneal Inflammation by Manumycins

Corneal inflammation can result from numerous types of eye injuries and medical procedures on the eye.[45] While Ras farnesyltransferase inhibitors are generally considered as potential anticancer drugs, [44] their ability to inhibit inflammation has also been documented.[35, 41, 42, 45] A study looking at two Ras farnesyltransferase inhibitors tested mice with corneal inflammation, that were induced by cauterization to the eye of each mouse, for the efficacy of the two compounds.[45] Manumycin A **1** and Gliotoxin were each dissolved in a 1 mM balanced salt solution containing 0.1 % Dimethyl Sulfoxide (**DMSO**). The inhibitors were then tested by applying the solution topically every 8 hours onto the eye with the induced corneal inflammation. It was found that these inhibitors have good therapeutic potential in inhibiting corneal inflammation that was induced by cauterization. As well, the study found that the two inhibitors selectively inhibited the macrophages at the cauterized cornea. Furthermore, Manumycin A **1** and Gliotoxin did not exhibit any discernible side effects on the cornea of the control mice used in the study.[45]

(1.6.2) Inhibition of Brain Tumours

At the University of Saskatchewan, researchers Wang and Macaulay compared the efficacy of Manumycin A **1** to Lovastatin on medulloblastoma cells *in vitro*.[46] Medulloblastoma is a malignant cerebellar tumour usually found in the brain of children 3 to 8 years old. Lovastatin is a competitive inhibitor of 3-hydroxyl-3-methylglutaryl coenzyme A (HMG-CoA), that previously exhibited inhibitory activity to medulloblastoma cells. Lovastatin induced cell apoptosis usually required 36 to 96 hours of treatment and caused cell cycle arrest. Whereas, the cells treated with Manumycin A **1** did not cause cell cycle arrest and cell apoptosis required only 12 to 24 hours of treatment. The authors of the study also questioned the importance of Ras inhibition in cell apoptosis when using Manumycin A **1**. As the authors noted, once Ras proteins are maximally inhibited by 10 μM of Manumycin A **1**, cell apoptosis can be increased by the addition of more Manumycin A **1**. They speculated that the inhibitor might be causing cell apoptosis through other biological pathways unrelated to Ras farnesylation.[46]

(1.6.3) Multiple Myeloma

Multiple Myeloma is a haematopoietic (formation of blood or blood cells) malignancy arising from the formation of blood plasma cancer cells (myeloma cells) that tend to collect in bone marrow.[47,48] When the myeloma cells collect at one site in a bone and form a tumour, it is called a plasmacytoma. Unfortunately, in most cases the myeloma cells collect in multiple sites on bones throughout the

body forming many plasmacytomas and this condition is called Multiple Myeloma. It is also important to note that there is a fine distinction between Multiple Myeloma and bone cancers. Multiple Myeloma is a cancer of the blood plasma immune cells that begin within the immune system but express tumours in bones; primary bone cancer instead starts within the bone. Multiple Myeloma is regarded as incurable and has demonstrated a strong resistance to standard chemotherapeutic strategies.

Researchers in Italy discovered the Interleukin-6 enzyme plays an important role in drug resistant myeloma cells.[47] The enzyme activates several cellular pathways for proliferation in opposition to the chemotherapeutic treatment. It was also noted that 40 % of Ras proteins in new Multiple Myeloma patients were mutated and as the disease progresses the amount of mutated Ras proteins also increased. *In vitro* treatments of myeloma cells with Manumycin A **1** stopped cell proliferation and induced cell apoptosis. Further analysis linked over expressed activation of caspase-3, an enzyme link to the cellular pathway of cell death, with the manumycin induced apoptosis. These results establish potential opportunities for additional research for a chemotherapeutic treatment of the fatal disease Multiple Myeloma.[47]

(1.6.4) Lung Tumours

In a study to test the efficacy of a number of Ras farnesyltransferase inhibitors on lung cancers, surprising results occurred when using manumycin A

1.[49] The study comprised two parts: an *in vivo* part against a lung tumour model in mice and an *in vitro* part involving four tumour cell lines. The *in vivo* evaluation of manumycin A **1**, gliotoxin, and dihydroepiandrosterone (**DHEA**) in the mice tumour model, showed the compounds increased tumour growth over the control mice in the study. Manumycin A **1** inhibits all four cell lines *in vitro* with an effective dose of 50% ($\mathbf{ED_{50}}$) at a 4-6 μM concentration. However, further testing *in vitro* demonstrated that when an insufficient concentration of manumycin A **1** was used (1μM), 3 of the 4 tumour cell lines are instead stimulated by 10-13 % more growth than the control cell line. Similar results were observed for the other two Ras farnesyltransferase inhibitors, gliotoxin and DHEA. The authors of the study speculated that perhaps the treatment *in vivo* may have increased a non-ras-dependent pathway for tumour growth when the effective concentration is too low.[49]

(1.6.5) Pancreatic Cancer

Pancreatic cancer is still resistant to most chemotherapeutic treatments and is the 5th leading cause of cancer death in the United States. Long term survival is only seen in patients who undergo complete resection of the localized tumour. A number of studies with manumycin A **1** have been undertaken to evaluate its efficacy in treating pancreatic cancer.[50-53] Pancreatic cancer is known to have a high (> 90 %) proportion of mutant K-Ras proteins. An *in vitro* study using human pancreatic cancers cells (SUIT-2, MIA PaCa-2, AsPC-1, BxPC-3) revealed that

manumycin A **1** inhibited all four types of pancreatic cancer cells in a dose-dependent manner. When the cancer cells were inoculated into nude mice, manumycin A **1** again inhibited tumour growth and also liver metastasizing cells.[51,52]

Presently, chemoradiotherapy is often used in the treatment of pancreatic cancer as it offers a slight improvement in the patient survival rates compared to other forms of treatment.[53] Manumycin A **1** was evaluated for increasing the radiosensitivity of pancreatic cancer xenografted onto nude mice. The results indicated that there is a substantial benefit for treating pancreatic cancer when incorporating Manumycin A **1** into chemoradiotherapy treatment.[53]

(1.6.6) Liver Cancer

Hepatocellular carcinoma is one of the most common lethal cancers in the world.[54-56] Presently, this liver cancer has no effective treatment and patients' long term survivability is poor and new therapies are badly needed. It previously has been reported that Hepatocellular carcinoma has N-Ras protein mutations and therefore, researchers investigated using Manumycin A **1** as a potential therapeutic agent. All three studies substantiated that Manumycin A **1** has an inhibitory affect *in vitro* and elicits the induction of apoptosis in HepG2 cells. Further analysis in the studies revealed that the HepG2 cells underwent DNA fragmentation after only 12 hours of treatment with manumycin A **1**. During induction of cell apoptosis by manumycin A **1** , p53 (a tumour suppressor protein) and $p21^{WAF1}$ (works

concurrently with p53) expression was increased. Also, the activation of caspases (the enzymes involved in cell death) and the inhibition of the NF-κB pathway (a defensive response mechanism pathway to stresses) were noted in the HepG2 cancer cells after treatment. The studies concluded that the use of manumycin A **1** shows potential as an effective treatment against hepatocellular carcinoma.[54-56]

(1.6.7) Colon Cancer

Various studies investigating the use of Manumycin A **1** in the treatment of colon cancer determined that the Ras farnesyltransferase inhibitor caused cell apoptosis in a number of different colon cancer cell lines.[57-59] In the colon cancer cell line LoVo, Manumycin A **1** exhibited a dose dependent inhibition of the cancer cells' proliferation *in vitro*. Further analysis showed that Manumycin A **1** also inhibited DNA synthesis.[57]

For the human colon cancer cell line COLO320-DM, which does not contain a mutant Ras protein, Manumycin A **1** was evaluated for its biological activity.[58] Manumycin A **1** was demonstrated to be cytotoxic to COLO320-DM cancer cells *in vitro*. The study also suggested that cell apoptosis is a result of the inhibition of the p21ras farnesylation, which reduced signal transduction through the p42MARK/ERK2 pathway (the p42MARK/ERK2 pathway is involved with growth factors of cells). The authors also noted that for cancer cells with non-mutant Ras proteins, less Manumycin was needed for cell apoptosis than when compared to studies with cancer cells with mutant Ras proteins. As a possible

reason for this difference, they suggested that cells with mutant Ras proteins are over expressed numerically, compared to cells with non-mutant Ras proteins. Thus cells with mutant Ras proteins need more biologically active compound to have the same results.[58]

A recent study evaluated the efficacy of Manumycin A **1** and BAL9611 (a geranylgeranyl-transferase inhibitor) against human colon (SW620) cancer cells.[59] The Ras superfamily of proteins are involved with cell proliferation in a number of different pathways which require post translational modifications to be biologically active. Two of the modifications to various Ras Proteins needed for post translational modifications include isoprenylations by farnesyltransferase and geranylgeranyl-transferase. BAL9611 is a novel inhibitor of geranylgeranyl-transferase which has exhibited cytotoxicity towards colon cancers. The results of the study determined that when Manumycin A **1** and BAL9611 were combined, they have a synergistic apoptosis effect on the SW620 cancer cells. This synergistic effect enables the induction of cell apoptosis to occur with reduced amounts of both inhibitors rather than when the two compounds are employed separately. These promising results were exhibited both *in vitro* and *in vivo*, with the *in vivo* testing taking place in CD nu/nu female mice xenographed with SW620 tumours.[59]

(1.6.8) Ovarian Cancer

Ovarian cancer affects thousands of women each year and is the most lethal gynaecological cancer.[60-62] In an effort to determine why ovarian cancer is so resistant to treatment if the initial therapy is unsuccessful, two studies examined the total proteins extracted from cancer cells treated with Manumycin A **1**.

In the two studies, it was discovered that soon after the treatment of resistant ovarian cancer cell lines (2774, OVCAR3, SFMAL) with Manumycin A **1,** heat shock proteins (**HSP**) 70 were up-regulated by the cancer cells.[60,61] The IC_{50} using Manumycin A **1** against all ovarian cell lines and cell cultures tested had an efficacy of between 3.7 μM to 25 μM. The researchers were able to isolate and identify HSP 70 and an altered form of HSP 70 after treatment with Manumycin A **1**. HSPs are a cellular defensive mechanism used to protect cells against damage that can cause necrosis or apoptosis. In the cells treated with Manumycin A **1,** HSP 70 were the only HSPs to be up-regulated. HSP 70 is known to react to various forms of cell stress in order to protect the cell from late stage cell apoptosis. An inhibitor of HSP 70, 50 μM of quercetin, was evaluated in combination with Manumycin A **1**. Quercetin significantly enhanced the efficacy of Manumycin A **1** towards ovarian cancer cells' apoptosis. These results provide the framework for a combinational "cocktail" for the treatment of ovarian cancers.[60-62]

(1.6.9) Mesothelioma Cells

Mesothelioma is a rare condition where a malignant tumour develops in the pleura or peritoneum and has been linked to exposure of asbestos fibre. Currently, there is no effective treatment for mesothelioma carcinoma. In the previously mentioned study on ovarian cancer, the researchers also examined the efficacy of Manumycin A **1** against mesothelioma cells (SFHAY, SFROB) cultured directly from patients and found the cancer cells to be significantly inhibited.[61] Similar to ovarian cancer, the researcher discovered an up-regulation of HSP 70 after a treatment with Manumycin A **1**. The authors of the study proposed that up-regulation of HSP 70 may be common to cancers resistant to standard chemotherapeutic treatments.[61]

(1.6.10) Anaplastic Thyroid Carcinoma

In North America there are about 15,000 new case of thyroid cancer per year, with three times more females being diagnosed with the disease than males.[63] Of the four types of thyroid cancer, 90 % of them are: Papillary, Follicular, mixed Papillary/Follicular or Hurthle cell. The prognosis for these common thyroid cancers is excellent with a better than 95 % cure rate. The last 10 % of thyroid cancers consist of Medullary and Anaplastic, with Anaplastic Thyroid Cancer being the rarest and most lethal.[63] Patients with Anaplastic Thyroid Cancer (**ATC**) have a mean survival time of 3-7 months once diagnosis is made for this very aggressive solid tumour.[64-68] The current therapeutic remedies include surgery,

radiotherapy and chemotherapy. All have had little effect on patient survival rates and consequently, there is an egregious need for new therapeutic treatments.

Researchers at the University of Texas (Houston) tested Manumycin A **1** alone and in combination with other drugs currently used to treat ATC.[63] Present chemotherapy uses Doxorubicin and Cisplatin to treat ATC, while the use of Paclitaxel is still under clinical evaluation. These compounds were investigated for their efficacy with Manumycin A **1** against six human ATC cell lines: ARO, C643, DRO, Hth-74, KAT-4, and KAT-18. The results of the study indicated Manumycin A **1** inhibited all six of the ATC cell lines *in vitro* in a dose dependent manner. Manumycin A **1** combined with Cisplatin or with Doxorubicin synergistically enhanced the cytotoxic effect for only five of the six ATC cell lines tested. However, when Paclitaxel was combined with Manumycin A **1**, the two compounds exhibited a synergistic enhancement of cytotoxic effect more than their simple addition against all six ATC cell lines *in vitro*. To confirm the existence of a synergistic effect for manumycin A **1** and Paclitaxel against ATC cell lines, the authors of the study used the median-effect method of Chou and Talalay. The ATC cell lines evaluated using this method were ARO, DRO, C643, and Hth-74. Two additional cell lines were included in the Chou-Talalay methods' evaluation, a pancreatic cancer cell line (PANC-1) and a breast cancer cell line (SK-Br3). The results of the evaluation indicated synergism for the combination of Manumycin A **1** and Paclitaxel against all six different cancer cell lines *in vitro*. To determine if the combination of compounds would cause toxicity to normal

cells *in vivo*, studies using the nude mouse xenograft model were conducted with ARO and KAT-4 cells. No indications of increased toxicity to normal cells or to the health of the host animal were observed while using Manumycin A 1 and Paclitaxel together. In the KAT-4 cells, the combination of compounds exhibited a greater antitumour response than the individual compounds alone, but synergism was not observed *in vivo*. Further testing is needed to optimize the delivery of the two compounds to maximize therapeutic efficacy.[64]

In an effort to understand the enhanced apoptosis of Anaplastic Thyroid Cancer by Manumycin A **1** and Paclitaxel, two studies investigated the activation of the apoptotic pathway in ATC cells by the two compounds.[67,68] The first study used inhibitors of caspase-9, caspase-8 and caspase-3, to determine that Manumycin A **1** and Paclitaxel initiated the apoptosis regulatory pathway at Cytochrome C or possibly even further upstream in the apoptosis pathway.[67]

The second study determined that p21 Waf-1 (also called: Cip-1, Sdi-1, MDA-6 factor) expression was linked to ATC Kat-4 cells susceptibility towards Manumycin A **1** and Paclitaxel.[68] By increasing the Waf-1 expression inside the ATC cells with a stable transfected plasmid that expressed p21 Waf-1 upon induction with Zn^{2+}, the authors were able to show that Kat-4 cells were more sensitive to the two compounds as Waf-1 increased. A second set of experiments used HCT-116 p21 knock-out (-/-) cells and wild type human colon cancer cells. The knock-out (-/-) cells expressing no p21 Waf-1 were able to proliferate after 24 hours of drug treatment with Manumycin A **1** and Paclitaxel. Whereas, the p21

wild type (+/+) human colon cancer cells were inhibited by the combination of Manumycin A **1** and Paclitaxel. Thus, expression of p21 Waf-1 is pro-apoptosis when ATC cells are treated with Manumycin A **1** and Paclitaxel.[68]

In the first study, tumours treated with Manumycin A **1** *in vivo* were observed to be paler than the control tumours suggesting a decrease in vascularity.[65] Angiogenesis, the process for new blood vessel formation, is a key factor in the continuous rapid growth of tumours. Without angiogenesis, tumours can only grow a few millimetres in size until they can develop an enhanced blood supply.[66] Therefore, a sustained delivery of Manumycin A **1** should increase the efficacy against the continuous process of angiogenesis for tumours.[65]

Matrigel implants in mice are a common method used to determine the extent of angiogenesis *in vivo*. Treatment of the xenograft ATC mice with the human ATC cell lines: ARO, KAT-4, KAT-18 and Hth-74, with two Matrigel implants per mouse were evaluated. Manumycin A **1** injections significantly inhibited angiogenesis as demonstrated by measurement of the decreased haemoglobin content, the reduced amount of endothelial cells and the vascularity within the Matrigel implants.[65] Vascular Endothelial Growth Factor (**VEGF**) is a mitogen (an agent that stimulates cell division) important to the process of angiogenesis. To discover if VEGF levels were being affected in ATC, the concentration of VEGF was evaluated for Manumycin A **1** and Paclitaxel individually and together in both tumour xenograft and tissue cultures of two ATC cell lines ARO and KAT-4. *In vitro* results showed Paclitaxel to exhibit a

negligible effect towards VEGF, but Paclitaxel did seem to enhance Manumycin A **1** efficacy towards VEGF. This same enhancement by Manumycin A **1** did not appear in the *in vivo* results for Paclitaxel. Manumycin A **1** did significantly inhibit VEGF in the two ATC cell lines *in vitro* and *in vivo*, agreeing with the results from previous studies using different farnesyltransferase inhibitors.[65]

Pharmacokinetics, the dynamic behaviour of chemicals inside biological systems, suggests that the manner in which a compound is administered can affect its therapeutic efficacy as well as the degree of toxicity of the therapeutic treatment.[65] As angiogenesis is a continuous process, slow release Manumycin A **1** pellets and injections of Manumycin A **1** were evaluated individually and in combination with Paclitaxel against the ATC cell line KAT-4 *in vivo*. The results indicated that a sustained delivery of Manumycin A **1** had an improved efficacy at a lower combined dose than the injected Manumycin A **1** *in vivo*. Also, sustained delivery of Manumycin A **1**, with or without Paclitaxel, exhibited no increase in its toxicity towards the mice with ATC. Thus, Manumycin A **1** and Paclitaxel inhibit angiogenesis in tumours and contribute to a significant antineoplastic (an anticancer drug) effect *in vivo* in addition to their direct cytotoxicity towards ATC.[65]

The potential of Manumycin A **1** for therapeutic treatments is exceptionally wide ranging in its possible future applications. Manumycin A **1** has shown promising results for antibiotic, antifungal, antiparasitic, anti-scarring, and antitumour properties. The antitumour properties of Manumycin A **1** show a

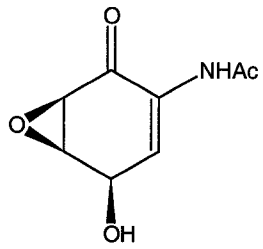
potential against some of the most virulent cancers that presently do not have viable treatments. The potential research value of Manumycin A **1** by itself or as a sophisticated cocktail should not be understated.

(1.7) Synthesis of Manumycins and Their Analogues

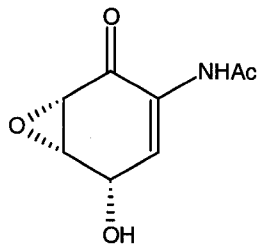
Due to the difficulty of producing cost effective quantities of manumycins from their bacterial source, the need for a novel synthesis of the *m*-C_7N unit is imperative to fully utilize the potential of all the 28 naturally occurring manumycins. Two main research groups having been working towards that endeavour. Richard J. K. Taylor leads a research group at the University of York and Peter Wipf directs another research group from the University of Pittsburgh. Both research groups have made initial progress towards discovering syntheses for some of the manumycins, but much work is still needed to fulfill the potential of manumycins.

(1.7.1) *m*-C_7N Core Analogues

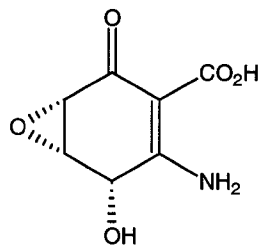
The epoxyquinone and epoxyquinol core is a common structural feature for many compounds isolated from natural products.[69] In addition to manumycins, a number of smaller secondary metabolites displaying structural similarities without the "eastern" and "southern" polyunsaturated chains typical of manumycins have been isolated and characterized.[69, 70] Representative examples of these secondary metabolites are found in Figure 2 and include: MT 35214 **3**, LL-C10037α **4**,



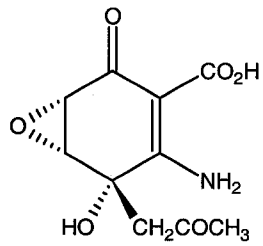
(3) (+)-MT 35214



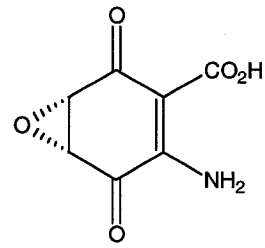
(4) (-)-LLC10037α



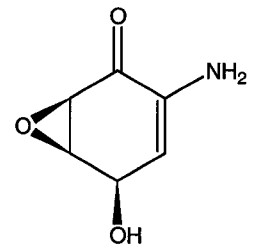
(5) (+/-)-Enaminomycin A



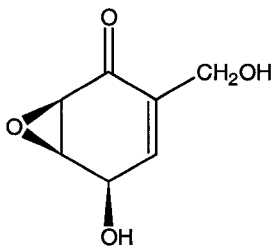
(6) (+/-)-Enaminomycin B



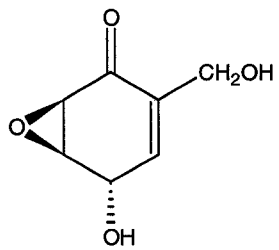
(7) (+/-)-Enaminomycin C



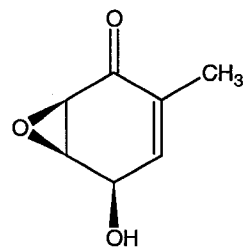
(8) (+)-MM14201



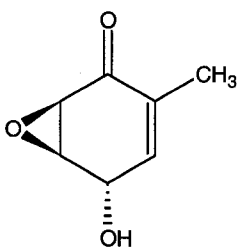
(9) (+/-)-Epoxydon



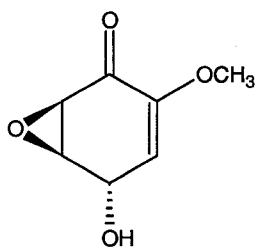
(10) **(+/-)**-Epi-epoxydon



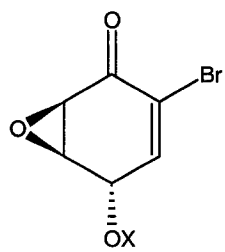
(11) (+/-)-Epoformin



(12) (+/-)-Epi-epoformin



(13) (+/-)-Chaloxone



(14) (+)-Bromoxone (X= H)
O-Acetylbromoxone (X = AC)

Figure 2 Biologically Active *m* - C_7N Core Analogues [11,70]

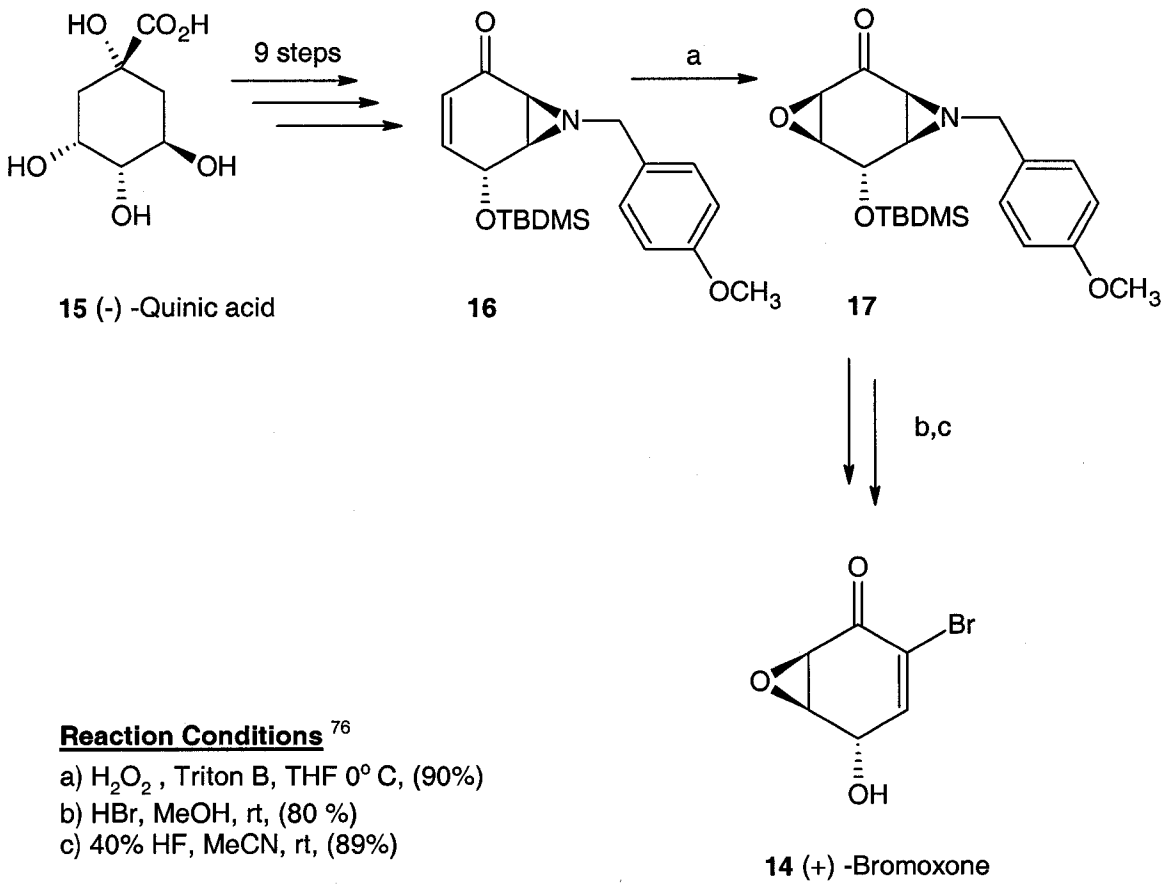
Enaminomycin A **5**, Enaminomycin B **6**, Enaminomycin C **7**, MM14201 **8**, Epoxydon **9** [11], Epiepoxydon **10**, Epoformin **11**, Epiepoformin **12**, Chaloxone **13**, and Bromoxone **14**.[70] Initial research into the total synthesis of manumycins was built upon previous attempts to synthesize some of these smaller metabolites analogous to the *m*-C_7N core. The secondary metabolites Bromoxone **14**, MT 35214 **3**, and LL-C10037α **4** were considered to be good analogues for examining potential stereoselective routes to various manumycins.[61]

(1.7.2) Synthesis of (+)-Bromoxone

(+)-Bromoxone **14** and its acetate derivative were first isolated from marine Acorn Worms (*Phyllum hemichordata*) found in deep sea caves off the coast of Maui.[71] Discovered by Higa and co-workers in 1987, the structures were confirmed using X-ray analysis of the acetate derivative. The acetate derivative exhibited good antitumour activity against P388 cells (a type of leukemia) with an IC_{50} of 10 ng mL^{-1} *in vitro*.

The synthesis of racemic (+/-)-Bromoxone was first reported by Taylor and co-workers in 1994.[72] An enantiomerically pure synthesis of (+)-Bromoxone **14** through way of enzyme resolution by Johnson's (1995) [73], by Altenbach's (2000) [74], and by Kitahara's (2003) [75] respective laboratories was also accomplished. Maycock *et al.* (2003) instead started with commercially available (-)-Quinic acid **15**, a compound from nature's chiral pool, which had the correct cyclohexane

Scheme 1



15 (-) -Quinic acid **16** **17**

14 (+) -Bromoxone

Reaction Conditions [76]

a) H_2O_2 , Triton B, THF 0° C, (90%)
b) HBr, MeOH, rt, (80 %)
c) 40% HF, MeCN, rt, (89%)

carbon skeleton and a 1,4 oxygen functionality suitable for synthesis.[76] (see Scheme 1) The (-)-Quinic acid **15** also already had the (4S)-hydroxyl functional group needed for the synthesis of (+)-Bromoxone **14**. Maycock developed a 12 steps synthesis from (-)-Quinic acid utilizing an aziridine functional group to protect the alkene and control the regiochemistry of the epoxidation of **16**. The aziridine functional group was introduced stereoselectively to produce **16** as the major diastereomer in a 4:1 ratio. It is believed that this functional group has the strongest orienting effect in the subsequent stereoselective formation of epoxide

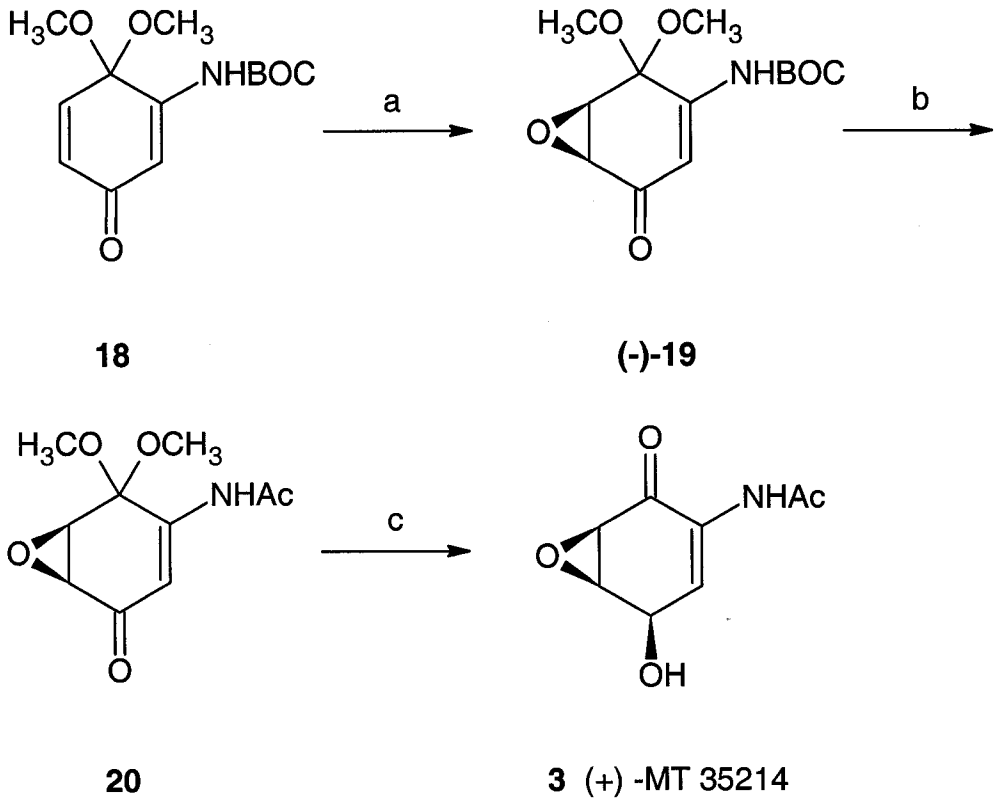
17. Originally, the tert-butyldimethylsilyl (**TBDMS**) functional group was thought to control the stereochemistry of the epoxides' formation due to steric hindrance. However, subsequent experimentation by the authors carried out on the minor deprotected product of **16** showed that it was the aziridine functional group that was directing the stereochemical outcome of the epoxidation. In this case, the minor diastereomer product was shown to have a syn (same side of the ring) configuration for the hydroxyl and epoxide functional groups after epoxidation. The minor isomer could therefore be used to pursue an asymmetric synthesis of LL-C10037α or manumycin analogues without the "lower" side chain.

(1.7.3) Synthesis of (+)-MT 35214

(+)-MT 35214 **3** is the enantiomer to (-)-LL-C10037α **4** and its m-C_7N core's stereochemistry is analogous to only two manumycins, Nisamycin and Alisamycin.[77] Taylor's research group had reported the only asymmetric synthesis of (+)-MT 35214 **3** , which was based upon the enantioselective chiral phase transfer epoxidation of an acetal protected quinone (see Scheme 2). Taylor's group utilized Wynberg's phase transfer epoxidation procedure using commercially available N-Benzylcinchonidinium chloride as the chiral catalyst to give compound (-)-**19** a 71 % yield from **18** and with an enantiomeric excess (**ee**) of 89 %. The total synthesis of **3** was completed in 7 steps from the commercially available starting material with an overall yield of 10 %. Taylor's research group also tried to use a pseudoenantiomeric N-benzylcinchoninium chloride to produce

the enantiomer (+)-**19** but surprisingly this catalyst also gave (-)-**19** in a lower optical yield of 10 % ee. The authors did not offer any explanation for this surprising result. Unfortunately, it was therefore concluded that this technique was unsuitable for the synthesis of (-)-LL-C10037α **4,** the enantiomer of (+)-MT 35214 **3.**

Scheme 2



Reaction Conditions [77]

a) t-BuOOH, NaH, N-benzylcinchoridinium chloride, (71%, 89 % ee) b) 1) CF_3CO_2H, (95%) 2) AcCl, t-BuOLi (65%) c) 1) $LiEt_3BH$ (89%) 2) Montmorillonite K10 (90%)

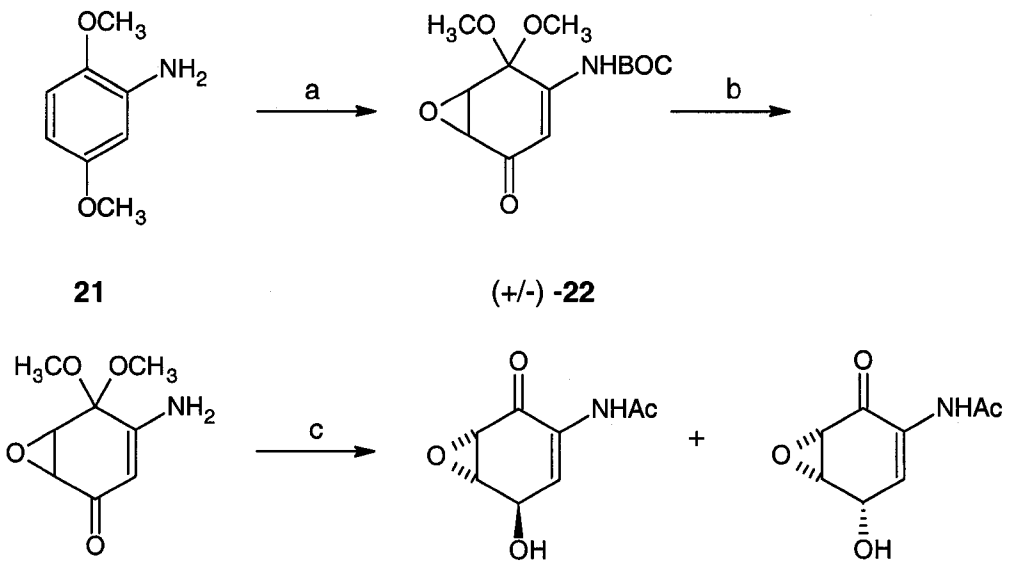
(1.7.4) Synthesis of (-)-LL-C10037α

The *Streptomyces LL-C10037* secondary metabolite (-)-LL-C10037α **4** was first isolated in 1984 by Lee and co-workers from Lederle Laboratories.[78] Its structure was later revised by Gould and co-workers based on the examination of a single crystal X-ray diffraction analysis and its stereochemistry was further confirmed by exciton circular dichroism studies of two derivatives of (-)-LL-C10037α **4** .[79] Gould and co-workers additionally proposed a biosynthetic pathway for (-)-LL-C10037α **4** based upon whole cell feeding with deuterated substrates and cell free studies.[80,81] The biosynthetic route consists of six additional steps from 3-hydroxyanthranilic acid derived from the Shikimic acid pathway.[81] The antibiotic antitumour (-)-LL-C10037α **4** metabolite exhibited inhibitory activity *in vitro* towards a limited number of Gram-positive and Gram-negative bacteria with MIC values ranging from 64 – 128 mg mL^{-1}. The compound (-)-LL-C10037α **4** also increased the life-span of mice with murine (relating to mice or rats) leukemia P388 by 29 % when intraperitoneal injections of an optimized dose were administered (25 $\mu g\ g^{-1}$). [78]

The synthesis of (-)-LL-C10037α **4** has been reported by various research groups in both racemic and enantiomically pure forms.[70] Wipf's [82] and then Taylor's [83] research groups both have reported a racemic synthesis for the two enantiomers, (+)-MT 35214 **3** and (-) LL-C10037α **4**, that are quite similar.[70] Taylor and co-workers were able to reduce the number of synthetic steps to 7 and

to increase the overall compound yield to 10 % from the same starting material, 2,5-dimethoxyaniline **21**, which was previously used by Wipf.[83] (see Scheme 3) Taylor first protected the amine functional group in **21** then oxidized using phenyliodine diacetate (**PIDA**) in methanol before epoxidizing the resulting

Scheme 3



Reaction Conditions[83]

a) 1) BOC_2O, THF, rt 2) PIDA, MeOH, 0° C 3) H_2O_2 / K_2CO_3, THF / H_2O, rt, 5 days
b) BF_3OEt_2, CH_2Cl_2, rt c) 1) AcCl, $LiOBu^t$, THF 2) $NaBH_4$ 3) TsOH, PPTs, aq. Acetone

intermediate with hydrogen peroxide and base to get compound (+/-)-**22**. To get to the key amine intermediate (+/-)-**23**, Taylor used boron trifluoride etherate to remove the t-butoxycarbonyl (**BOC**) protecting group. Compound (+/-)-**23** was acetylated and the carbonyl was reduced to the corresponding alcohols with sodium borohydride. Hydrolysis of the dimethoxy acetal afforded a 1:3

diastereomeric mixture of (+/-) **24/25** and their diastereomers (+)-MT 35214 **3** and (-)-LL-C10037α **4**. The separable 3:1 mixture of the diastereomers afforded a 10 % yield for the racemic target enantiomer compounds (+)-**3** and (-)-**4**.

The first enantioselective synthesis of (-)-LL-C10037α **4** was reported by Wipf *et al.* in 1995.[84] However, the use of multiple protecting groups, chiral auxiliary and two hypervalent iodine oxidations, resulted in a very low overall yield for this synthesis.

Later, an enantioselective synthesis of (-)-LL-C10037α **4** was reported by Johnson *et al.* [85] and Altenbach *et al.* [74], where both research groups used enzyme resolution of an intermediate to afford the stereoselectivity needed to isolate the enantiomer (-)-LL-C10037α **4**.

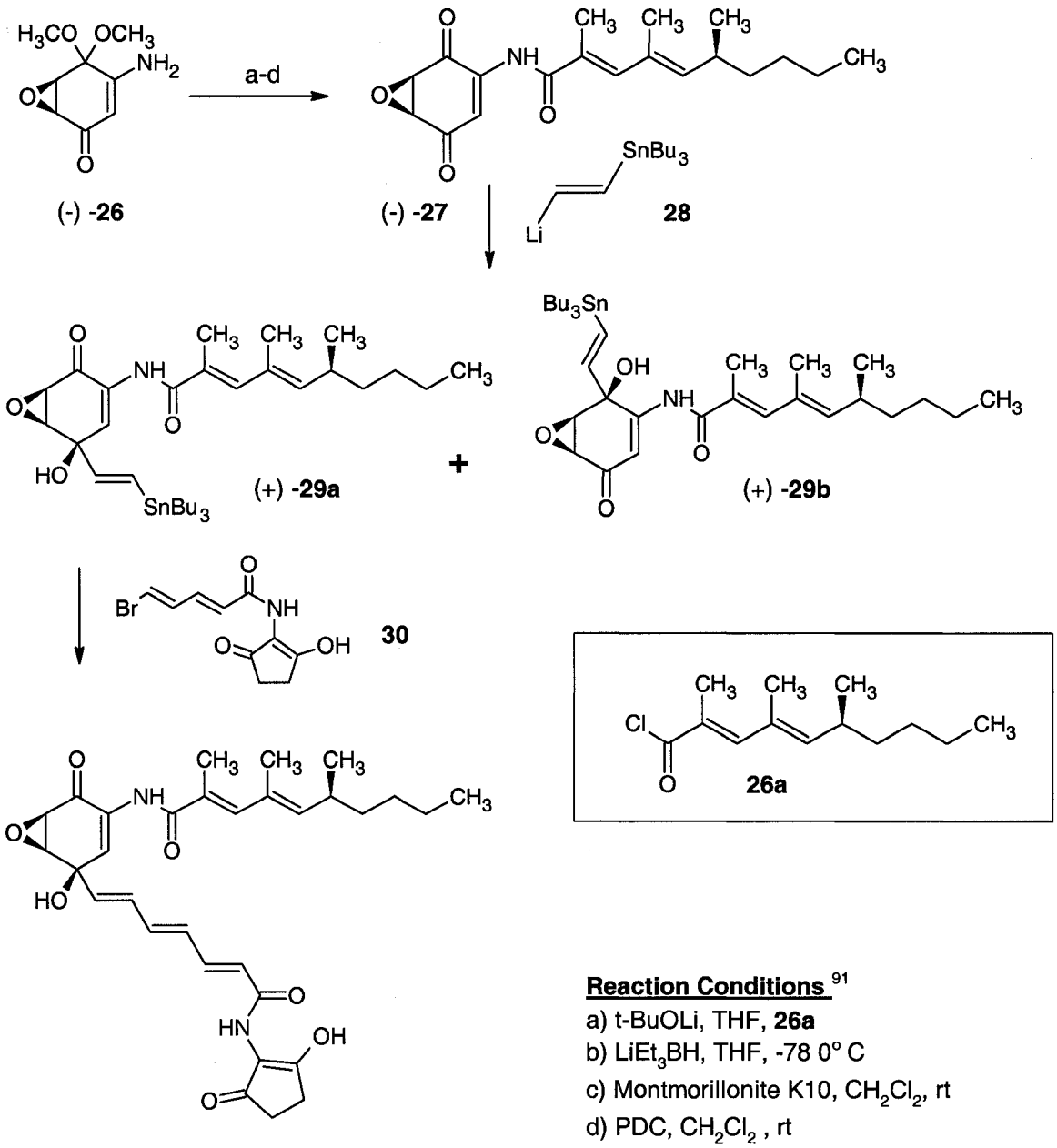
(1.7.5) Total Synthesis of Manumycins

The total synthesis of a number of racemic and enantiopure manumycins have been accomplished by Taylor *et al.* They published the first manumycin to be synthesized, (+/-)-Alisamycin in 1996, using the methodology to make the *m*-C_7N unit derived from their earlier work with (+/-)-Bromoxone, and (+/-) LL-C10037α.[86] Taylor and co-workers were soon to follow with the racemic synthesis of other manumycins: (+/-)-U-62162 [87], (+/-)-Nisamycin [88] and (+/-)-Colabomycin D.[89] Wipf *et al.* also published a synthesis for (+/-)-Nisamycin in

1999, based on their methodology developed for the *m*-C_7N unit in their synthesis of (+/-) LL-C10037α.[90]

The first enantioselective synthesis of a manumycins was published by Taylor *et al.* in 1998.[91,92] They reported the synthesis of (+)-Manumycin A **31**, the enantiomer of the natural product (-)-Manumycin A **1.** Using their methodology developed for the synthesis of (+)-MT 35214 **3**, (see Scheme 2) Taylor was able to stereoselectively make the protected quinone (-)-**26** using Wynberg's chiral phase transfer catalysis procedure. (see Scheme 4) The next step was the addition of the "eastern" polyunsaturated side-chain **26a** (see box in Scheme 4) using an acid chloride derivative and then deprotection of the acetal to afford compound (-)-**27** in a moderate yield (68 %). To add the "southern" polyunsaturated side chain, Taylor utilized a two step procedure where first the vinyl-lithium reagent **28**, was reacted with the quinone (-)-**27** stereoselectively, but not regioselectively to afford compounds (+)-**29a** and (+)-**29b**. After isolating compound (+)-**29a**, the final step was a Stille coupling where (+)-**29a** is united to the bromo diene derivative **30** using Negishi's catalyst to afford the target molecule (+)-Manumycin **31**. Based on this work, the previously published structure of the natural product (-)-Manumycin A **1** was revised to the new syn configuration for the epoxide and hydroxyl group found on the natural product.

Scheme 4



(-)-Alisamycin was the next manumycin to have a total stereoselective synthesis published by Taylor *et al.* in 1998.[77] Using the same methodology as they used to prepare (+)-Manumycin **31**, Taylor was able to easily extend their

previous research to make the natural product (-)-Alisamycin. The only change needed was to use an acid chloride derivative with the appropriate polyunsaturated "eastern" side chain fitting the structure of (-)-Alisamycin. Contrary to their previous synthesis of (+)-Manumycin where the enantiomer of the natural product was obtained, (-)-Alisamycin shares the same *m*-C_7N core's stereo-configuration as (+)-MT 35214 **3**, therefore, the synthesis of the natural product (-)-Alisamycin was possible rather than its enantiomer.[77]

In a continuation of their work on the total synthesis of manumycins, Taylor *et al.* developed a methodology for converting type I manumycins into their corresponding type II manumycins.[93] A number of the type II manumycin natural products discovered are just a reduced form of some type I manumycin natural products. The four type II manumycins, called TMC-1 A-D (see Appendix 2), also exhibit cytotoxic activity to a range of tumour lines *in vitro* and structurally have a β-hydroxy ketone configuration on their *m*-C_7N core unit. By regioselectively reducing the corresponding analogous type I counterpart's epoxy ketone *m*-C_7N core unit, Taylor was able to synthesize (+)-TMC-1 A from the reduction of (+)-Manumycin B with sodium phenylseleno(triethoxy)borate (**Na [PhSeB(OEt)$_3$]**). Taylor additionally reported the first synthesis of (+)-Manumycin B the enantiomer of the natural product (-)-Manumycin B, using their Wynberg chiral phase protocol for the *m*-C_7N core unit.

(1.8) Spirocompounds

Spirocompounds are a large class of inorganic and organic compounds that consist of multiple rings linked through one common atom. The nomenclature and the name spirane was first reported by A. Baeyer in 1900 for bicyclic compounds with one common atom to both rings.[94] An interesting sub-category of this large class of compounds are the cyclic spiro-tyrosine metabolites isolated from a number of different natural sources. Some examples of this class of compounds includes: Aranorosin **32**, Aranosinol A **33** and B **34**, Aranochlor A **35** and B **36** and Gymnastatins A **37**, B **38**, C **39**, D **40**, E **41** and I **42**, as shown in Figures 3 and 4.

(1.8.1) Aranorosin Family of Compounds

Aranorosin **32** was first isolated from the fermentation broth of *Gymnascella dankaliensis* (formerly named: *Pseudoarachniotus roseus*) and has been shown to exhibit positive biological activity towards a variety of fungi, bacteria and cancers on a micromolar scale *in vitro*.[95] Aranorosin **32** has an unusual 1-oxaspiro[4.5]decane ring system, (see Figure 3) its total synthesis has been reported by the two research groups of Wipf [96,97] and Taylor.[98] Since its initial discovery in 1988, further Aranorosin like spiro-Tyrosine metabolites have been isolated and characterized.

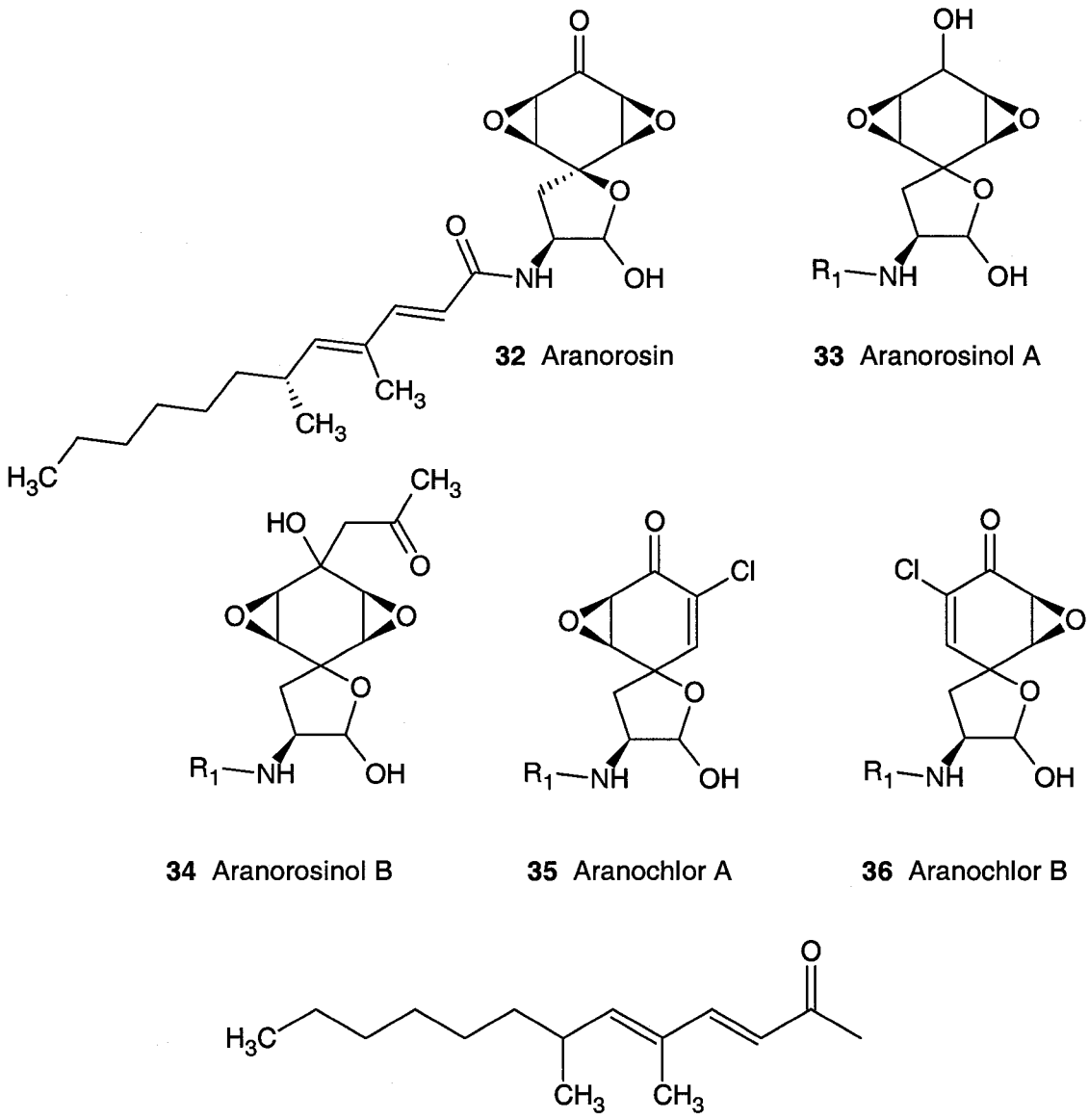


Figure 3 Aranorosin Family of Compounds[98-101]

The spiro-tyrosine secondary metabolites Aranorosinol A **33** and B **34** were isolated in 1992 from a strain of *Pseudoarachniotus roseus*.[99] Both compounds contain the 1-oxaspiro[4.5]decane ring system of Aranorosin **32** and exhibited *in vitro* inhibition of an assortment of bacteria and fungi in the μg mL^{-1} range. A

more recent examination of the biological properties of Aranorosinol A **33**, showed the inhibition of POLO-like kinase 1 (**Plk1**) enzyme with a MIC of 118μM.[100] Plk1 is a highly conserved kinase enzyme that has been revealed to be over-expressed in cancer cell lines and has an essential role in cell regulation. Therefore, Plk1 is also a potential anti-cancer target for cancer research which is inhibited by a compound containing a 1-oxaspiro[4.5]decane ring system.

Another study looking for solutions to the rapid emergence of antibiotic resistance in pathogenic bacteria found and isolated Aranorosinol B **34** from a screening of 4000 microbes.[101] Aranorosinol B **34** is a potent inhibitor against autophosphorylation of YycG, an essential histidine kinase in the stress-response pathway in bacteria. Comparative experiments against the established antibiotics cefazolin, amikacin, vancomycin, erythromycin and ofloxacin determined negligible inhibition of *Bacillus subtilis* YycG. Whereas, Aranorosinol B **34** inhibited YycG from both *Bacillus subtilis* and *Staphylococcus aureus* with an IC_{50} of 223 and 211 μM respectively. Thus Aranorosinol B **34** inhibits a biological pathways in bacteria current antibiotics do not utilize and therefore could be developed to be used against antibiotic resistance in pathogenic bacteria.

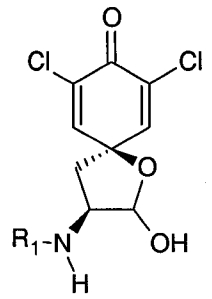
Two new additions to the Aranorosin family of compounds includes the isolation of secondary metabolites Aranochlor A **35** and B **36** in 1998 from *Pseudoarachniotus roseus*.[102] Both metabolites contain the 1-oxaspiro[4.5]decane ring system of the other aranorosins and also inhibit a variety of bacteria and fungi *in vitro* in the mg mL^{-1} range. What is new to the aranorosin type carbon skeletons

of both aranochlors is the addition of a chloroalkene in place of one of the epoxides. With the incorporation of a vinyl halide functional group into their structure, Aranochlor A **35** and B **36** posses a strong structural similarity to the spirocyclized gymnastatins, a family of compounds with potent cytotoxicity towards various cancers.[100,102]

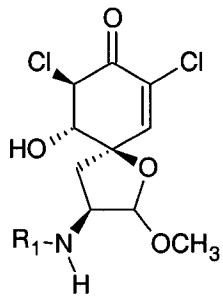
(1.8.2) Gymnastatins Family of Compounds

In a search for new antitumour secondary metabolites from the marine environment, researchers in Japan isolated a number of biologically active compounds from the fungus *Gymnascella dankaliensis*.[103] The fungus was discovered residing on the marine sponge *Halichondria japonica* which researchers were able to separate from the sponge and cultivate. After 4 weeks of growth in a salt water buffered medium, a variety of cytotoxic secondary metabolites were isolated from the fungus using multiple separation techniques to afford the following types of compounds: dankasterone , gymnasterones and gymnastatins.[103–106] Most of the metabolites examined exhibited moderate to high biological activity towards cultured p388 lymphocytic leukemia with an ED_{50} in the $\mu g\ mL^{-1}$ to $ng\ mL^{-1}$ range. (see Figure 4)

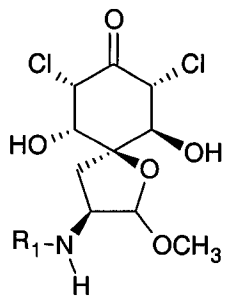
The Gymnastatins A-E **37-41** and Gymnastatin I **42** [100] contain the 1-oxaspiro[4.5]decane ring system of the aranorosin family of compounds.[106] The total synthesis of Gymnastatin A **37** and Gymnastatin I **42**, along with various analogues have been accomplished and have been used to establish the absolute



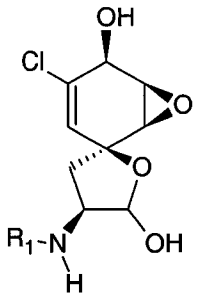
37 Gymnastatin A



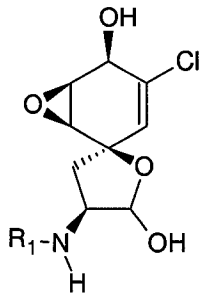
38 Gymnastatin B



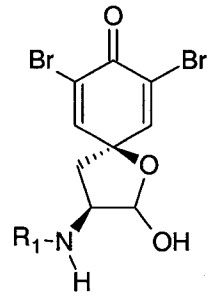
39 Gymnastatin C



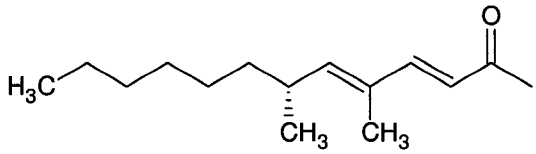
40 Gymnastatin D



41 Gymnastatin E



42 Gymnastatin I



R_1 is the polyunsaturated chain for all 6 Gymnastatins

Figure 4 Gymnastatin Family of Spirocyclic Compounds[100,106]

stereochemistry of most of the compounds in this class.[100] To date all the gymnastatins and aranorosins share the same 6R configuration at the 4,6-dimethyl-dodecadiene-2E,4E-oic acid unit attached to the nitrogen. The cytotoxic activity of Gymnastatins A-E **37-41** against cultured p388 lymphocytic leukemia was ED_{50}

0.018 μg mL (**37**), 0.108 μg mL (**38**), 0.106 μg mL (**39**), 10.8 μg mL (**40**) and 10.8 μg mL (**41**) respectively.[106]

(1.9) Cyclohexadienone ketals and quinols

In synthetic organic chemistry many biologically active compounds targeted for synthesis share carbon skeleton fragments bearing similar functional groups that are often referred to as building blocks. Over time, some of these building blocks become highly developed intermediates repeatedly used by organic chemists for the unique regioselective and/or stereoselective controls that they afford. Cyclohexadienone ketals and quinols are examples of building blocks useful to chemists for their potential to develop enantioselective syntheses of natural products.[107] Between the two types of cyclohexa-2,5-dienones commonly used as building blocks for the synthesis of natural products, the masked *para*-benzoquinone ketals are more enantioselectively developed when compared to the *para*-quinols. (See Figure 5) Presently, there are a number of enantioselective syntheses using the masked *para*-benzoquinone ketals as building blocks to generate the *m*-C_7N unit for some members of the Manumycin family of compounds. Additionally, masked *para*-benzoquinone ketals have also been used to generate enantioselective syntheses for the phenol Miroestrol, the ketals Preussomerin and Diepoxin, and the quinoid Jesterone. The generalized approaches developed to control the para-benzoquinone ketals' enantioselectivity have included the applications of enantioselective reagents, enantioselective

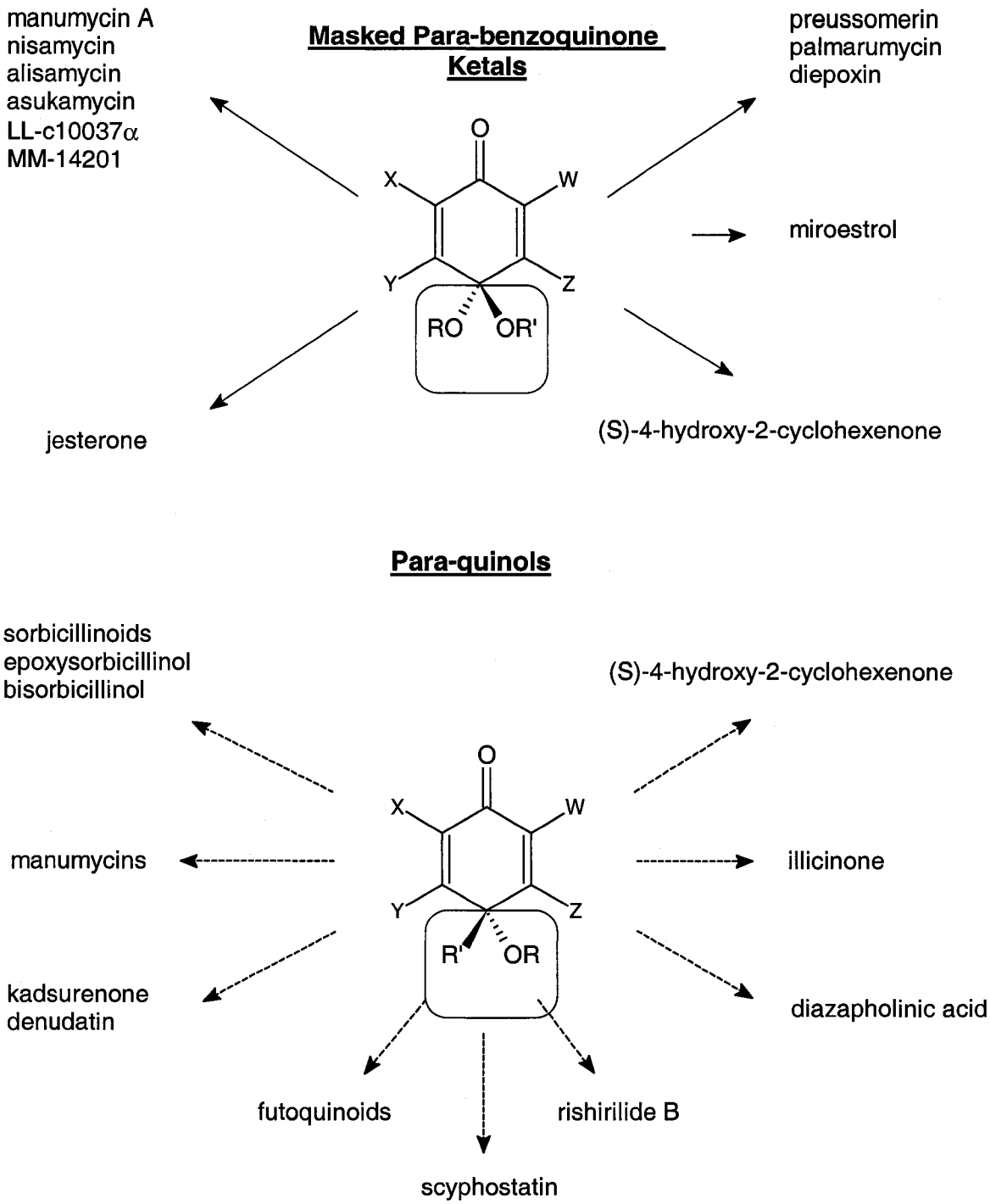


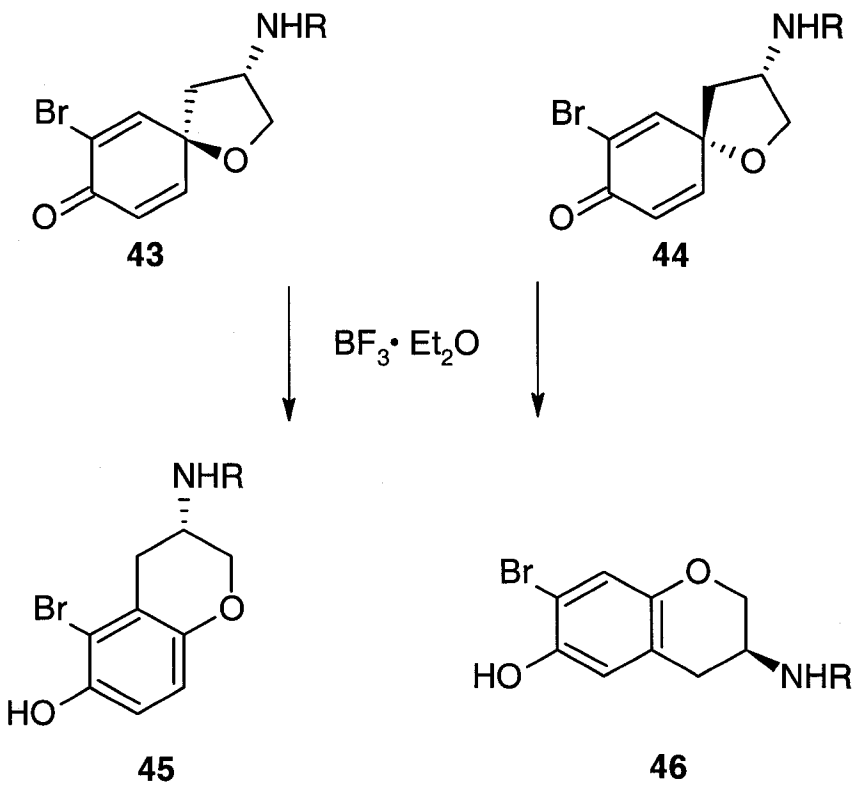
Figure 5 Enantioselective Syntheses using Masked *Para*-benzoquinone Ketals and Plausible Enantioselective Syntheses using *Para*-quinols.[107]

catalysts, and chiral auxiliaries. On the other hand, the *para*-quinols have not been extensively used in the synthesis of many natural products in contrast to the masked *para*-benzoquinone ketals. The unrealized potential for enantioselective applications of *para*-quinols could increase dramatically if the lack of synthetic pathways to nonracemic *para*-quinols could be solved. Currently, only Aranorosin and a few gymnastatins have been enantioselectively synthesized using L-Tyrosine, an amino acid from the chiral pool and a major building block of those compounds. The development of a convenient methodology to produce enantioselective *para*-quinols could facilitate the synthesis of a number of important biologically active compounds as was illustrated in Figure 5.[107]

(1.10) Development of *Para*-quinols Enantioselectivity

While there are many reports dealing with diastereoselective reactions involving *para*-quinols, a survey of the literature shows a lack of research concerning the enantioselective formation of *para*-quinols.[107] The following examples of enantioselective control involving *para*-quinols illustrate some of the limited research that has been developed thus far. Nishiyama *et al.* discovered that the spirocompounds **43** and **44** when treated with the same Lewis Acid created different benzopyrans **45** and **46**. (see Scheme 5) While this technique does not afford enantioselective formation of a *para*-quinols it does afford diastereoselective product resolution.[108]

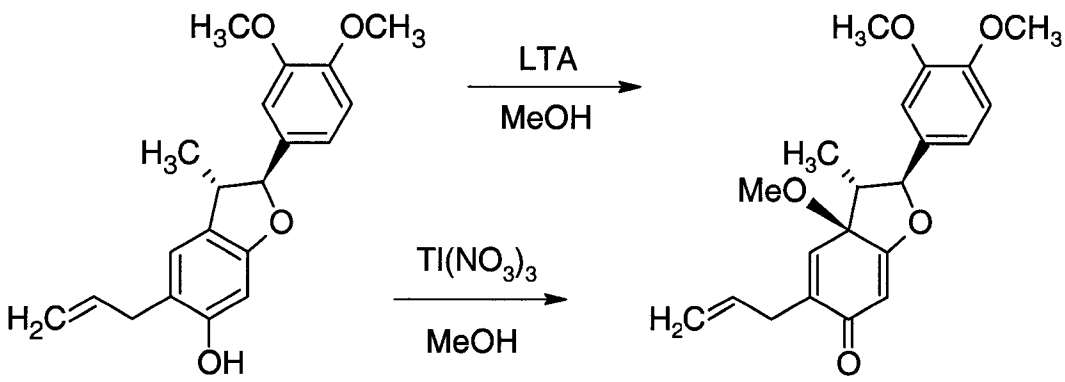
Scheme 5



An example of stereocontrol in the formation of *para*-quinols can be found in the work of Ponpipom *et al.*.[109] (See Scheme 6) They discovered by using different oxidants such as Lead (IV) acetate **(LTA)** versus Thallium (III) nitrate **(TTN)**, that different diastereoselective ratio of products could be obtained. Ponpipom's synthesis for Kadsurenone **48** using TTN gave only the anti-isomer product (as shown in Scheme 6) due to the oxidant's reaction mechanism and the steric effect of a methyl group located on the furan ring of the substrate. However, when the same starting material is treated with LTA, diastereoselective control is lost due to LTA's reaction mechanism being different from TTN and is no longer influenced by the methyl group on the residue. This loss of diastereoselective

control now afforded a product in a 1:1 ratio of syn and anti-isomers when using the oxidant LTA.

Scheme 6



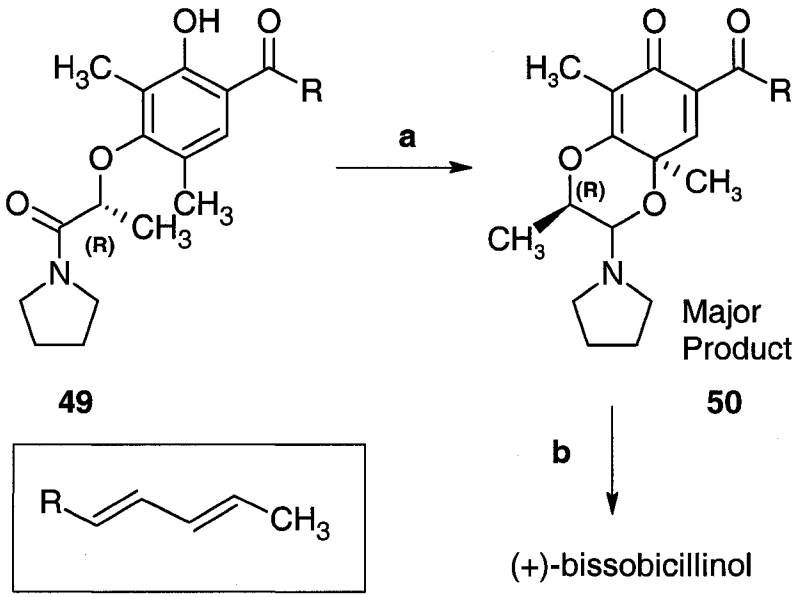
47 **48** Kadsurenone

Furthermore, using the oxidant TTN does illustrate excellent diastereoselective formation of *para*-quinols, this technique is unfortunately very substrate specific and cannot be considered applicable to a large variety of substrates.

A more interesting synthetic strategy recently reported by Pettus in two articles involved the use of a tethered chiral chain to induce remote stereoselectivity for the formation of a pair of different *para*-quinols.[110,111] In the first report, Pettus used an (S)-lactic amide tethered chain attached to a phenolic derivative to diastereoselectively produce the *para*-quinol **50** in a 3:1 ratio.[110] (where the major diastereomer is shown in Scheme 7) After chromatographic separation of the *para*-quinols, and further modifications to the appropriate *para*-

quinol, this work led to the enantioselective synthesis of (+)-bisorbicillinol in greater than 99% ee.

Scheme 7



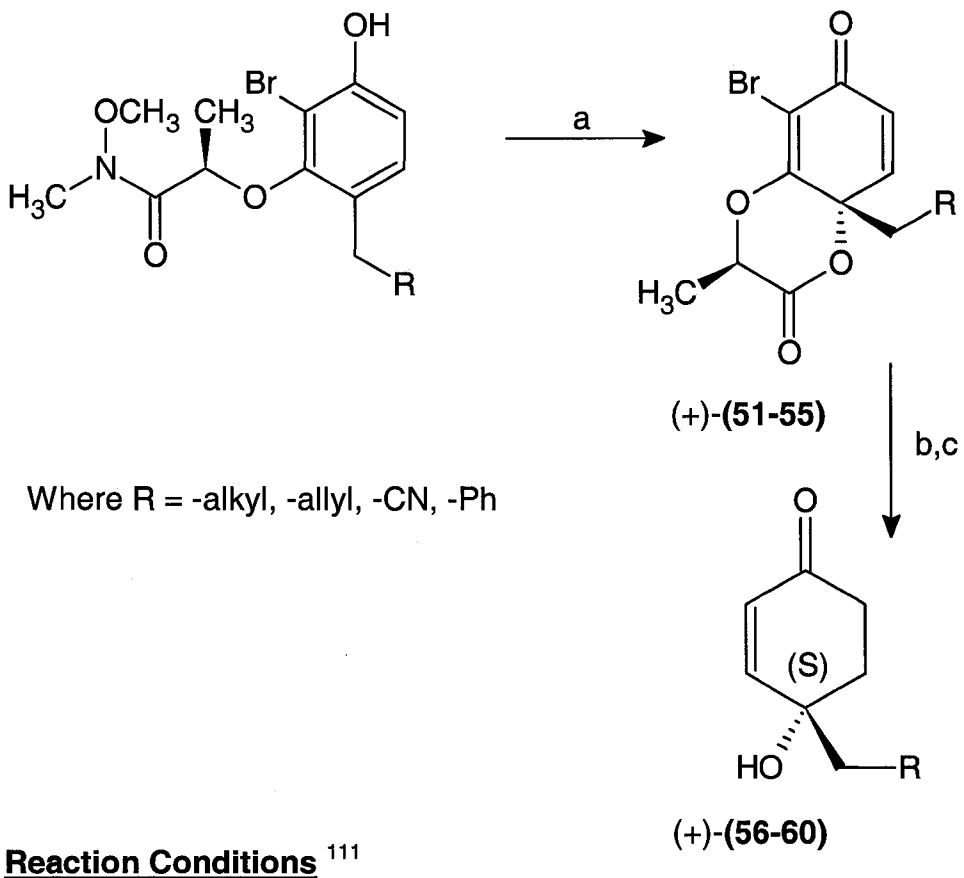
Reaction Conditions [110, 111]

a)PIFA, CH_2Cl_2/CH_3NO_2 (3:1) b) 1) conc. HCl, THF
2) KOH, H_2O 3) H_3O^+ work-up

Pettus then expanded on his original premise and found a new tethered amide chain that could induce a diastereoselectivity of 20:1 for the oxidative cyclization of the phenolic derivatives (**51-55**) into *para*-quinols.[111] A subsequent reduction and an elimination of the directing amide group afforded the chiral cyclohexenols (+)-(**56 –60**) with greater than 99% ee. (see Scheme 8)

In an analogous synthetic strategy to Pettus, Plourde was able to show a diastereoselective spiroannulation from an assortment of racemic phenolic derivatives.[112] (see Scheme 9) Unlike when Pettus added a chiral auxiliary to the starting material that had to be removed later, Plourde incorporated the chiral

Scheme 8



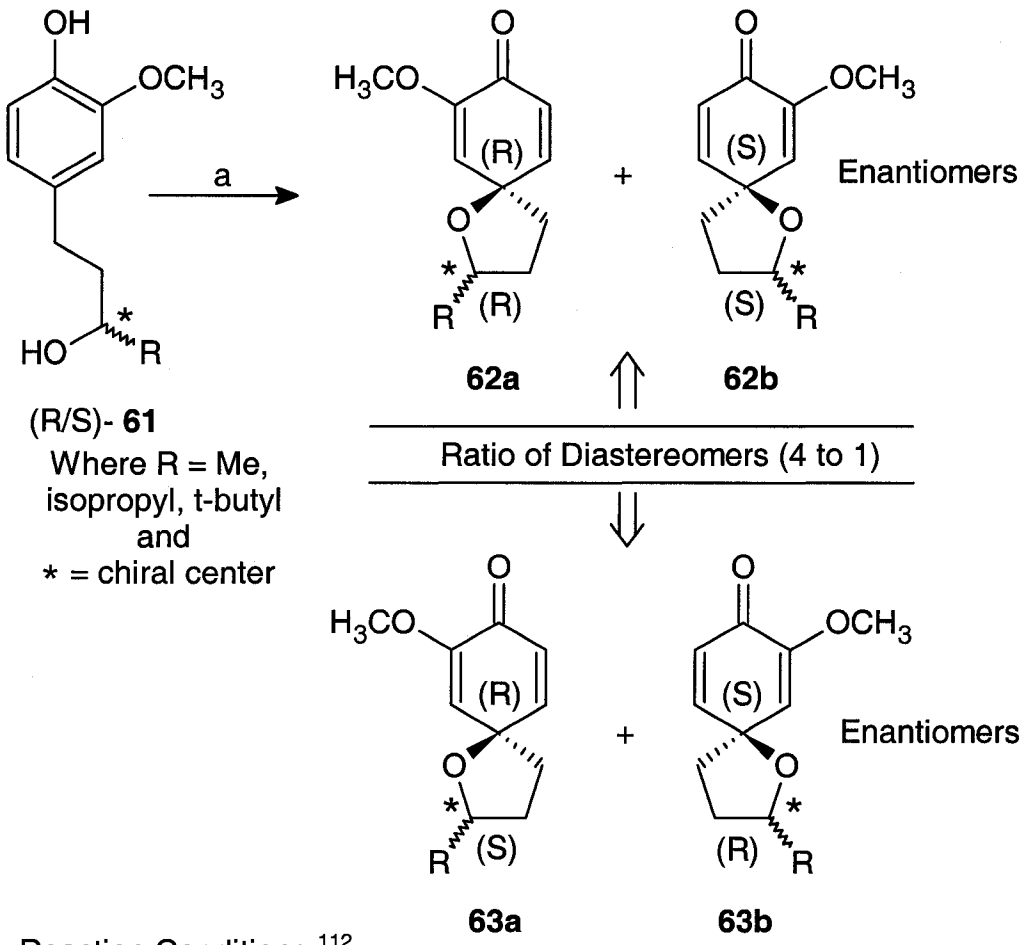
Reaction Conditions [111]

a) 1) PIFA, CH_2Cl_2/CH_3NO_2 (1: 2.5) 2) H_2O b) PtO, H_2, MeOH

c) KOTMS, Et_2O

centre directly onto the tethered *para*-alkyl residue of the phenolic derivatives. Plourde then utilized an intramolecular oxidation reaction with the tethered chiral chain to direct the diastereoselective formation for a number of racemic *para*-quinols. By starting with compound **61**, a racemic mixture of R and S enantiomers, the oxidative spiroannulation created four stereoisomeric products giving two pairs of enantiomers, **62a/b** and **63a/b**. The ratios for the two groups of diastereomers **62** and **63**, increased to a 4:1 ratio as the R group on the derivatives increased in size from a methyl group to a t-butyl group. Since **62** and **63** are

Scheme 9



diastereomers, their ratios could be determined by comparing the integration of ^{1}H-NMR spectra of the crude products. The synthesis shows that the tethered chiral chain induces a facial selection in the oxidative spiroannulation forming these spiroether compounds.

In Scheme 9, the stereoconfiguration of the chain is not assigned to provide a simple representation of the four stereoisomers. Unfortunately, sometimes the Cahn-Ingold-Prelog rules cause a change in designation when changing R groups,

without a corresponding change of inversion of the stereocenter. This occurs in Scheme 9, when representing the change from the methyl group to the two other functional groups, isopropyl and t-butyl. Therefore, the chiral tethered chain's stereocenter is not represented and is only labelled to help represent the four different stereoisomers.

(1.11) Concluding Remarks

A review of the literature shows that there are two distinct problems with using the benzoquinone methodology for the synthesis of natural products. The first problem is a lack of stereoselective facial control for the synthesis of epoxyquinones and the second problem is a loss of regioselective control when attempting to connect the southern chain to the *m*-C_7N unit of manumycins. This thesis is a study directed towards the diastereoselective formation of the manumycin *m*-C_7N core using the quinol methodology. We believe that the pursuit of a simple solution to the regioselective control is to use a starting material that already has an alkyl chain attached to the ring. Similarly, the issue of facial stereoselectivity for epoxidation reactions is addressed by the use of a spirolactone intermediate. A spirolactone intermediate with a 1-oxaspiro[4.5]decane configuration has previously exhibited an inherent stereoselective control for syn epoxidation reactions. The final problem of facial selectivity will be solved by using a chiral tethered chain to direct diastereoselective facial control. We believe that by using the quinol methodology this will address the problems associated

with the benzoquinone methodology. Thus enabling us to produce the core *m*-C_7N units stereoselectively for a majority of the manumycin natural products.

Chapter 2

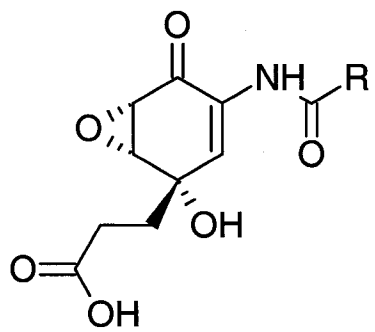
Results and Discussion

(2.1) A Novel Diastereoselective Methodology for *Para*-quinols

An interest in developing synthetic strategies for controlling the facial selectivity for the asymmetric formation of spirolactones and spiroethers, led us to investigate and develop a synthetic method using a chiral tethered chain. The significance of these types of *para*-quinols is their potential to be used as building blocks for the asymmetric synthesis of many natural products. In the course of examining previously published methods for the formation of spirocompounds, it was observed that the aranorosin and gymnastatin families of compounds shared many structural features with the central core *m*-C_7N unit of the compounds from the manumycin family. This similarity was especially apparent if the spiroether/lactone moiety was imagined to be opened. From this observation, a plan was developed to incorporate our ongoing interest in asymmetric spiroannulations into an approach to create a stereoselective analogue for the core *m*-C_7N unit of the manumycin family of compounds. As was previously mentioned in Chapter 1, the manumycin compounds exhibit a vast range of potential applications in many areas of research and medicine yet, currently only one *para*-quinol stereoisomer can be made proficiently.

Therefore, the ultimate goal of our research group is to synthesize the simple idealized target molecule **64** (see Figure 6) that represents the

stereoselective core *m*-C_7N unit for a majority of the manumycins. However, first we needed to determine the validity of using a tethered chiral chain to induce diastereoselective control for the formation of spirolactones. The simple target molecule **64** was therefore devised to include the specific criteria relating to



64

Figure 6 The Target Molecule
m-C_7N unit analogue

the proposed methodology of an asymmetric spiroannulation from a (L)-Tyrosine derivative. By utilizing a (L)-Tyrosine derivative from the chiral pool of amino acids, we afford ourselves the ability to quickly test whether employing a tethered chiral chain would induce the desired diastereoselective control for the formation of the spirolactone residues. An additional benefit to using an (L)-Tyrosine derivative is that the (D)-Tyrosine isomer is available commercially and therefore affords the opportunity to produce both *para*-quinol enantiomer based solely on choice of starting material.

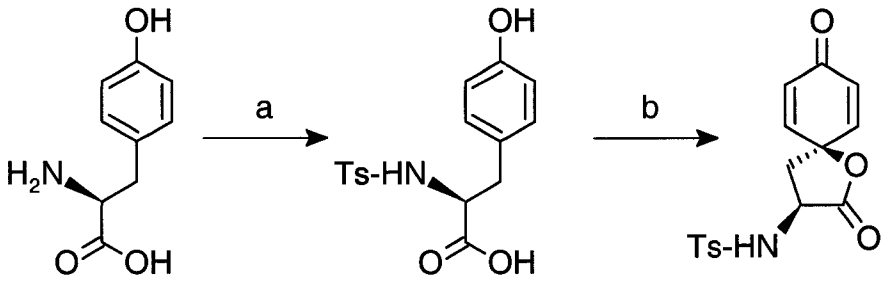
(2.2) Test Reactions for the Spiroannulation of (L)-Tyrosine

The first step in the process for the development of the asymmetric synthesis was to confirm that using a new substituent, Toluenesulfonyl **(Ts),** on the amino functional group of (L)-Tyrosine would not negatively affect the oxidative spiroannulation reaction.[95] Typically, spiroannulation of Tyrosine has been done with other substituents (N-Ac, N-Cbz, N-BOC, N-phthalamido) and to our knowledge this is the first recorded synthesis using the N-Ts substituent. Therefore, a number of test reactions using (L)-Tyrosine with a Ts residue attached to the amino functional group were carried out. Additionally, these test reactions allowed us to further optimize the reaction condition for the spiroannulation with the new type of starting materials.

Initial attempts for selective tosylation of only the amino functional group on the Tyrosine amino acid were problematic. The innate nature of the Tyrosine compound is for tosylation to occur at both the amino and the phenolic hydroxyl functional groups concurrently. There are varying strategies in the literature involving multi-step synthesis with up to two different types of protecting groups for adding a substituent to the Tyrosine compound's amino functional group.[113, 114] However in 2001 Ciufolini *et al.*, reported a three pot synthesis for the formation of (N-Ts)-Tyrosine **65** [115-117] but, in an effort to utilize an efficient total synthesis a novel two pot synthesis was developed to produce (N-Ts)-Tyrosine **65** (See Scheme 10). Instead of combating Tyrosine's propensity for quantitative di-tosylation with tosyl chloride, [118] we alternatively cleaved the tosylate back into a

hydroxyl group forgoing the need for any additional reaction steps with protecting groups altogether.[119] The different reaction conditions needed to cleave the tosyl group from the amino moiety [120] on the (L)-Tyrosine compound led to simplification of the synthetic procedure for the formation of (N-Ts)-Tyrosine **65**.

Scheme 10



L-Tyrosine **65** **66**

Reaction Conditions

a) 1) TsCl Et_2O, 1 M NaOH 2) KOH EtOH, 80-85 ^{0}C

b) PIDA, PIFA or LTA in Acetone at 0 ^{0}C - rt

The di-tosylation of L-Tyrosine proceeded smoothly with the formation of a white suspension. The work up in the literature cited was temperamental [118] so it was modified to an acidification with hydrochloric acid (pH 1-2), followed by the extraction of the aqueous mixture with ethyl acetate to afford the crude intermediate di-tosyl-tyrosine. The crude product was then selectively detosylated with potassium hydroxide in ethanol overnight at 85-90 ^{0}C. Compound **65** was obtained as a white solid in a 92 % yield from the starting material (L)-Tyrosine after silica gel chromatography.

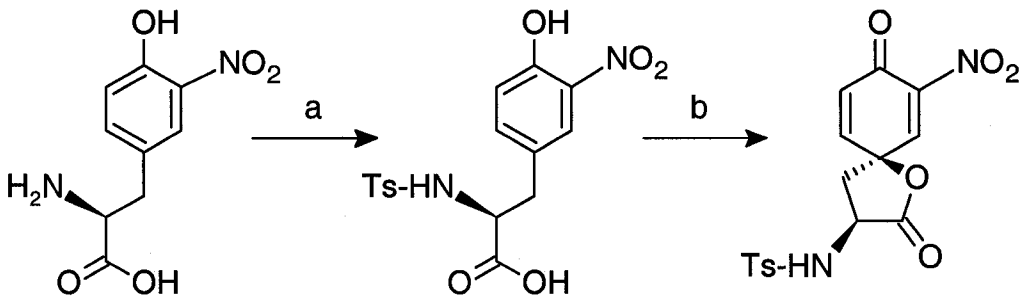
After the synthesis of compound **65,** the oxidative spiroannulation reactions were evaluated on small scale using various reaction conditions with three different oxidants (Phenyliodine (III) diacetate (**PIDA**), Phenyliodine (III) bis (trifluoroacetate) (**PIFA**), Lead (IV) acetate (**LTA**) [121-124]) and were found to proceed to the (N-Ts)-Tyrosine spirolactone **66** with no unexpected complications. The PIFA in acetone reaction was repeated on a larger scale and purified for analytical information and afforded **66** a 36 % yield. It should be noted, that since these were test reactions to ascertain the effect of the Tosyl group on the spiroannulation of the (L)-Tyrosine derivative, compound **66** was not fully characterized and assignments of the structures were based solely on the ^{1}H and ^{13}C NMR spectra of the purified product. As well, the spirolactone **66** does not contain a new chiral centre at the spirocarbon, so we proceeded with the next series of reactions using the 3-Nitro-Tyrosine as starting material.

(2.3) Spiroannulation of (L)-3-Nitro-Tyrosine Derivative

To evaluate the premise that a tethered chiral chain could direct diastereoselectivity we proceeded with spiroannulation of the 3-Nitro-Tyrosine tosyl protected derivative. Formation of the (N-Ts)-3-Nitro-Tyrosine derivative **67** using the test reactions conditions from Scheme 10 occurred much more slowly and with a lower yield (25-54 %). However, it was found that by substituting tetrahydrofuran (**THF**) for diethyl ether the reaction rate was increased along with the total isolated yield for compound **67** to 86% (See Scheme 11). The

spiroannulation of compound **67,** unlike the test reactions for the spiroannulation of compound **65** did not proceed as expected. While thin layer chromatography (**tlc**) seemed to suggest the reaction was proceeding

Scheme 11



L-3-Nitro-Tyrosine **67** **68**

Reaction Conditions

a) 1) TsCl THF, 1M NaOH 2) 1M KOH EtOH, 80-85 °C

b) PIDA, PIFA or LTA in Acetone at 0 °C - rt

correctly and a ^{1}H-NMR spectrum of the crude product exhibited trace amounts of the expected product, purification using silica gel chromatography did not allow us to isolate the spirolactone **68.** After many futile attempts using a myriad of reaction conditions and purification techniques, we re-examined the spirolactone **66** product for possible problems. It was decided that the nitro functional group being such a strong electron withdrawing group (**EWG**) at the 3-postion on compound **67**, might be interfering with the oxidative spiroannulation reaction.[125-127] To determine a possible solution to the problem, new test reactions were

devised to confirm that an electron donating group (**EDG**) would rectify the problem.

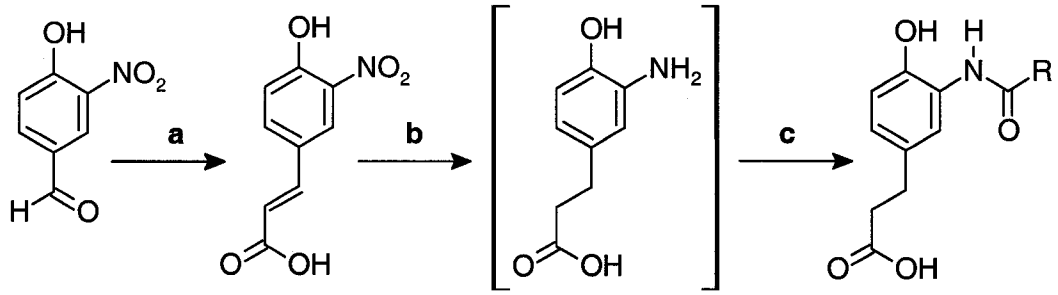
(2.4) Spiroannulation With An Electron Donating Group Derivative

The new test reactions consisted of a simplified non-Tyrosine version of (N-Ts)-3-Nitro-Tyrosine **67** but bearing an EDG instead of an EWG. To make the starting material, a Knoevenagel condensation with the Doebner modification was used to extend the aldehyde on 4-Hydroxy-3-nitrobenzaldehyde to a propenoic acid moiety as shown in Scheme 12, giving **69** in 93 % yield. Compound **69** could be used without purification.[128, 129] Hydrogenation transformed the nitro functional group to an amine while also reducing the alkene on the para-chain to a propanoic acid residue.[130] Due to compound **70** decomposing quickly with handling, the amine was directly converted into two different types of amides with either acetyl chloride or benzoyl chloride, making the new EDG's at the 3-position of the benzene ring for compounds **71** and **72** with yields of 48 % and 83 % from **69**.[131]

With compounds **71** and **72** in hand, we proceeded with testing whether the oxidative spiroannulation would proceed as expected with an EDG. The small scale oxidative spiroannulation of the derivatives **71** and **72**, using the reaction conditions used in our first test reactions, (1 to 3 equivalents oxidant, in acetone at 0 ^{0}C) proceeded with good yields for both compounds using all three oxidants as determined by ^{1}H-NMR spectra of the crude products (see Scheme 13). Thus, confirming our suspicions that the strong electron withdrawing nature of the nitro functional group was the cause of our previous difficulties with the

Scheme 12

4-Hydroxy-3-nitrobenzaldehyde



69 **70** **71** R= CH_3

72 R= Ph

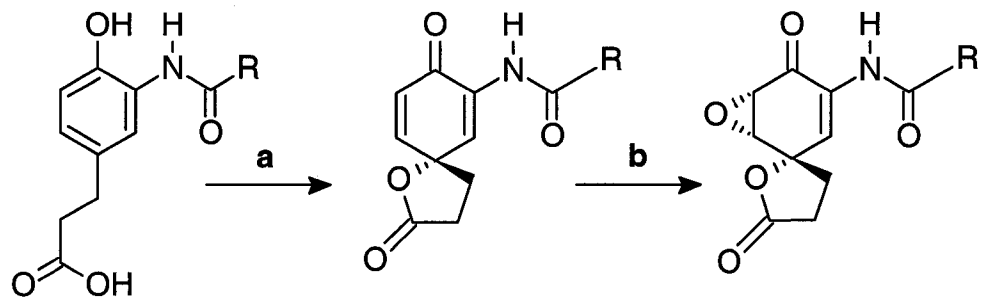
Reaction Conditions

a) 1)Malonic acid, pyridine, piperidine, 60-65 ^{0}C, 2) H_3O^+ b) H_2, Pd/C, THF

c) AcCl or BzCl , THF, rt

spiroannulation of (L)-3-Nitro-Tyrosine derivative. The reactions with **71** and **72** using PIFA in acetone were repeated on a larger scale and purified giving 96 % and 86 % yields for (+/-)-**73** and (+/-)-**74** respectively.

Scheme 13



71 R= CH_3 (+/-)-**73** R= CH_3 (+/-)-**75** R= CH_3

72 R= Ph (+/-)-**74** R = Ph (+/-)-**76** R=Ph

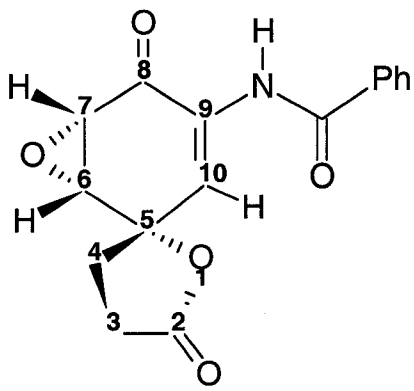
Reaction Conditions

a) PIDA, PIFA, or LTA, Acetone, 0 ^{o}C - rt b) H_2O_2 /$NaHCO_3$ [3:1] THF/ H_2O

The amide spirolactones (+/-)-**73** and (+/-)-**74** both contain a racemic mixture of enantiomers and share a spirolactone carbon skeleton with the Aranorosin (see Figure 3) and Gymnastatin (see Figure 4) families of compounds. Furthermore, assuming that the electron poor alkene (or least substituted alkene functional group) of compound **73** and **74** can be selectively epoxidized, the novel compounds **75** and **76** would represent racemic analogues of the *m*-C_7N unit's carbon skeleton found in the manumycin family (see Figure 2) and would be one reaction away from being a *m*-C_7N unit.

To show that it was possible to control the regio and stereoselective epoxidation of these spirocompounds **73** and **74**, we examined the hydrogen peroxide reaction on the racemic mixture of compound **74**. The un-optimized treatment of **74** with hydrogen peroxide and sodium bicarbonate in THF and water, led to the epoxidation of the electron poor alkene on **74** giving compound (+/-)-**76** in a low isolated yield of 7 %. [132-137] The regioselective aspect of the epoxidation on the electron poor alkene was confirmed by a Distortionless Enhancement by Polarization Transfer –135 (**DEPT-135**) NMR spectrum. The two carbons of the epoxide ring with one attached hydrogen each (a methine group, (**CH**)), showed two CH signals in the DEPT-135 NMR spectrum at 51.5 ppm (C-6) and 55.4 ppm (C-7). These signals were in the range one would expect to see epoxide CH signals (40-80 ppm from the internal standard of tetramethylsilane (**TMS**)) (See Figure 7 and page 115 in Appendix 1). Conversely,

if the epoxide had formed on the electron rich alkene (or more substituted alkene) there would have been only one CH signal, as C-9 is a quaternary carbon and does not have hydrogen attached. The stereoselective aspect of the syn epoxidation, between the oxirane and the lactone's oxygen, also has literature precedent for nucleophilic reactions occurring with high π-facial selectivity towards the alkene on the cyclohexadienone ring and to proceed with the syn configuration with respect to the lactone oxygen.[95, 138-141] Hence we assume that the epoxidation will produce (+/-)-**76** with the relative stereochemistry as shown in Figure 7, although we were unable to prove this assignment as of yet.



(+/-)-**76**

Figure 7 Compound (+/-)-76 with the proposed relative stereochemistry and labeled carbons for the DEPT-135.

Now that we confirmed the feasibility of our synthetic method to synthesize the racemic carbon skeleton analogues of the *m*-C_7N unit of the Manumycin family of compounds, we returned our attention back to using a chiral tethered

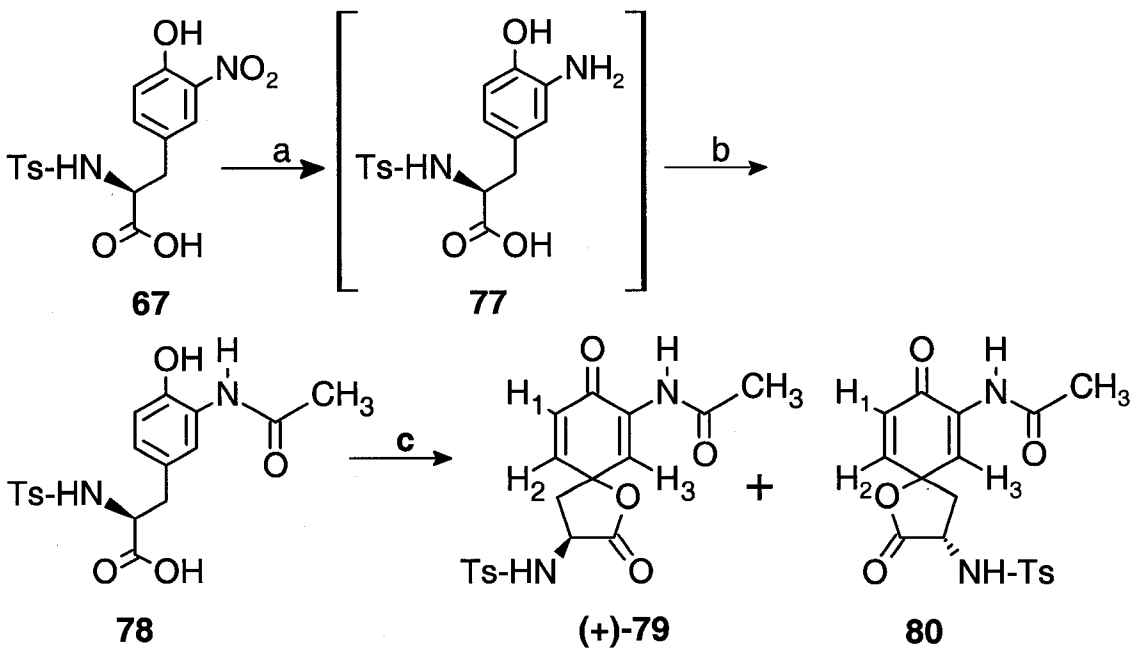
chain in order to ascertain the possibility of controlling the diastereoselective formation of the spirolactones.

(2.5) Diastereoselective Formation of Spirolactones

The diastereoselective formation of the spirolactones using a tethered chiral chain was accomplished using the EDG Tyrosine derivates **78** to make the spirolactone compounds (+)-**79** and **80** (see Scheme 14). By taking the information learned from the EDG test reactions study we were able to successfully complete the spiroannulation of compounds (+)-**79** and **80**. The previously prepared compound **67** (Scheme 11) was reduced to the amine **77** using hydrogen with a 10 % palladium/carbon catalyst. Due to instability problems with purifying the crude product, compound **77** was reacted directly with AcCl to form the amide **78** in a 59 % yield for the two reactions. With an EDG at the 3-position on the benzene ring of the Tyrosine derivative, the small scale oxidative spiroannulation reactions with the three oxidants proceeded as expected using the methodology from the first test reactions study (1 to 3 equivalents oxidant, in acetone at 0 ^{0}C). It is clear from ^{1}H-NMR spectra of the crude products that we now have a mixture of diastereomers. The use of an EDG on the 3-position of the aromatic ring for compound **78** solved the previous problem of the spiroannulation reactions and allowed the formation of the diastereomers with selectivity. Using the integration

of the ^{1}H-NMR spectrum signals for H_1 , H_2 , and H_3 of the crude products **79** and **80** (see Scheme 14) we were able to estimate the ratio of diastereomers. The ^{1}H-NMR spectrum peaks' integration ratios for H_1 (6.25 ppm major - 6.30 ppm minor diastereomer), H_2 (7.06 ppm major – 6.92 ppm minor diastereomer), and H_3 (7.52 ppm major – 7.66 ppm minor diastereomer) were compared and gave us a major to minor ratio of [3:1] for compound (+)-**79** and **80** respectively (see pages 125-128 in the Appendix 1 for expansions of ^{1}H-NMR spectra).

Scheme 14



Reaction Conditions

a) H_2 , 10% Pd/C, in THF b) AcCl, THF, rt

c) PIDA, PIFA, or LTA, Acetone, rt

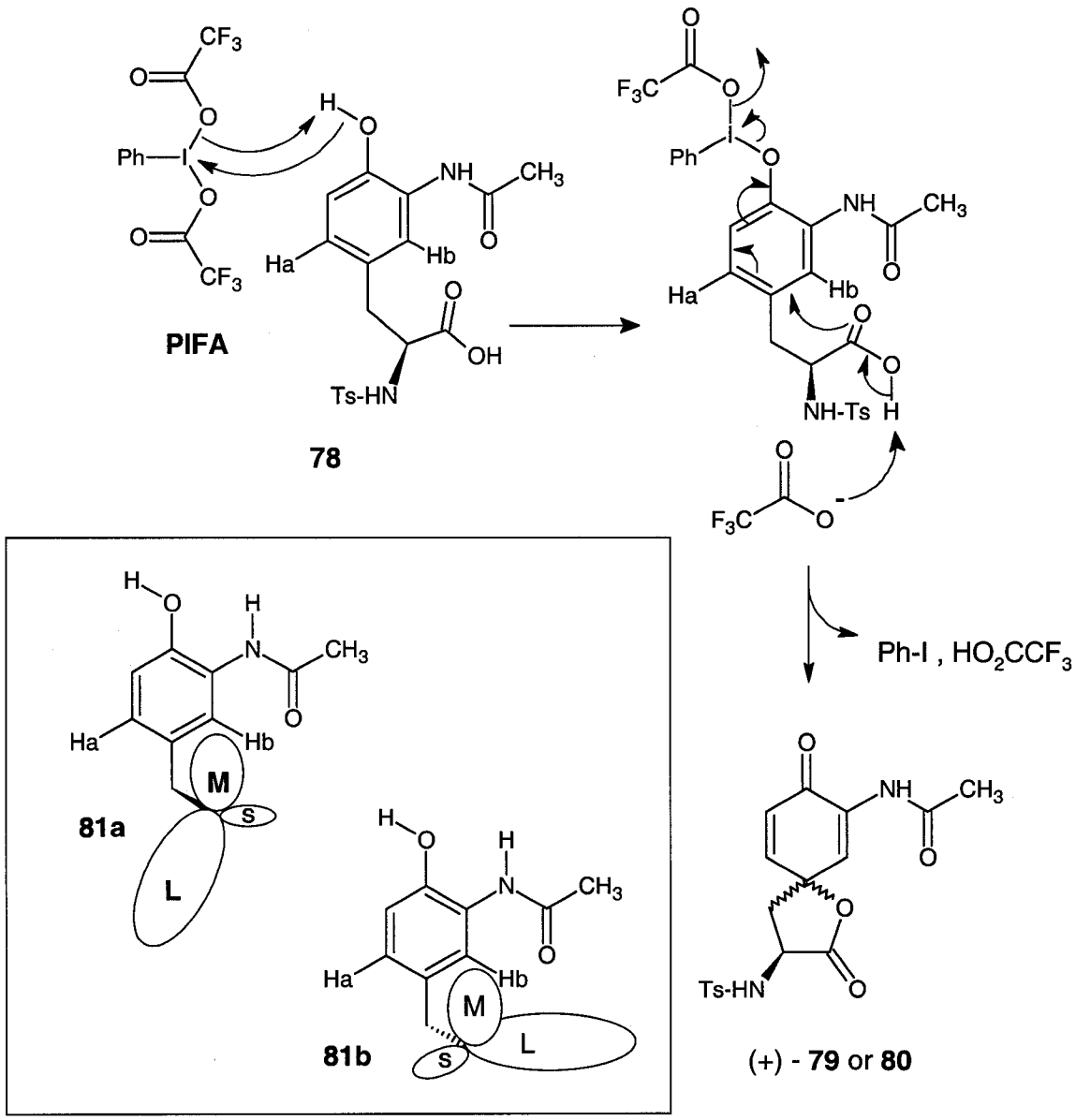
However, after separating the two diastereomers (+)-**79** and **80** by silica gel chromatography we obtained a higher ratio of major and minor diastereomers (using their isolated masses, 18 mg: 1 mg). A larger scale reaction using PIFA as

the oxidant, gave a silica gel chromatography separation of 38 mg for the major diastereomer, 54 mg for a mixture of major and minor diastereomers, and only 8mg of the minor diastereomer (total yield of 85 %). While the mass-to-mass ratio does not agree with the ^{1}H-NMR integration ratio, there is precedent in the literature for spirolactones to react with silica gel and this could be the cause for the discrepancy.[112, 127] Another possibility is one of the diastereomers may decompose at a different rate with the silica gel than the other diastereomer.

Scheme 15 shows the proposed reaction mechanism for the PIFA oxidant reacting with compound **78** in order to prepare the two diastereomers as shown above. PIFA reacts with the phenolic hydroxyl creating a hypervalent iodine complex. The conjugate base deprotonated the carboxylic acid which initiated the spiral annulation of the hypervalent iodine complex creating the two diastereomers. The structure **81** in Scheme 15 represents the anticipated diastereomeric transition state where the labels S, M and L represent the functional groups and their size: S for hydrogen, M for carboxylic acid and L for toluene sulfonylamide. The proximity of the tethered chiral chain to the aromatic ring limits the free rotation of the chiral center and the chiral center's interaction with the hydrogen protons Ha and Hb on the aromatic ring causes the resulting 3 to 1 diastereoselectivity. We suspect that the transition state of **81a** leads to the major isomer (+)-**79**. In this configuration the large sulfonylamide group orients itself on the side of the proton Ha where steric factors are less important than the transition

state **81b**. In the transition state **81b** a model study shows a larger steric hindrance between the sulfonylamide, the proton Hb, and the acetamide functional groups, thus leading to the minor diastereomer **80**.

Scheme 15



Chapter 3

Conclusions

(3.1) Racemic Analogues

The syntheses from Scheme 12 and Scheme 13 in Chapter 2 illustrate a convenient method to incorporate the "Eastern side chains" from any of the 28 Manumycins into the starting material for oxidative spiroannulations. The new synthetic route allows us to create a racemic core *m*-C_7N unit analogue for each manumycin natural product. This novel methodology allows for starting from the commercially available 4-Hydroxy-3-nitrobenzaldehyde and five steps later assembling the carbon skeleton of the m-C_7N core unit. With this methodology we can incorporate any of the 28 manumycin "Eastern side chains" to make new analogues (as shown in compound **83** and compound (+/-) –**76** in Figure 8 and Scheme 13, respectively). These new analogues will allow for further biological research related to their possible medicinal properties. Please note, as was previously mentioned in the introduction in sections 1.5.1, 1.5.2, 1.5.3 and 1.5.6 racemic manumycin benzoquinones also exhibited similar biological activities as their natural product counterparts. Consequently the "Southern side chain" is believed to be non-essential for certain Manumycin biological activities. As a result, this method allows for the synthesis of new compounds not of the benzoquinone **80** morphologies but of the *para*-quinol **81** configuration. Thus

expanding on previous research and creating new compounds that could exhibit similar or greater biological activity based on their structural similarity to the *m*-C_7N core units (Figure 2), to the aranorosins (Figure 3), to the gymnastatins (Figure 4), and to the manumycin families of compounds (Appendix 2). An additional advantage to creating analogues with this procedure is the high regio and modest stereoselective control afforded to the formation of the oxirane versus the racemic formation of the benzoquinone oxiranes. The *para*-quinol analogue **81,** having the syn configuration between the oxirane and the spirolactone oxygen, could possibly improve the biological activity of these compounds compared to the racemic benzoquinone analogue **80**. Subsequent biological testing will now allow for the determination of the importance for this structural feature for manumycin analogues.

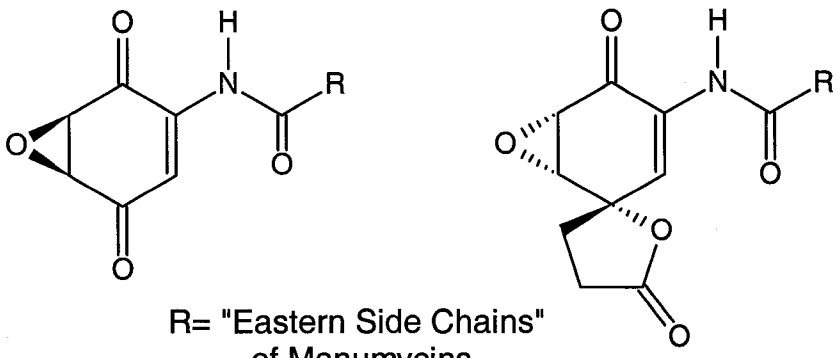


Figure 8 Comparing Benzoquinones to *Para*-Quinols

(3.2) Diastereoselective Analogues

The use of a tethered chiral chain on the (L)-Tyrosine derivatives in Scheme 14 is a viable method for inducing diastereoselective formation of *para*-quinol intermediates. The oxidative reaction produced a major and a minor diastereomeric spirolactone ratio ((+)-**79** and **80**, 3:1 respectively), thus confirming a facial discrimination caused by the tethered chiral chain. Similar to the research by Pettus [110,111] and Plourde [112], the use of a tethered chiral chain has exhibited a preference for the formation of a specific diastereomer. While the previous racemic formation of spirolactones allowed us to create racemic *m*-C_7N core unit analogues, the use of a tethered chiral chain now allows for the formation of two diastereomer *m*-C_7N core unit analogues that are amenable to flash column chromatography. The ability to separate these two diastereomers potentially allows for future synthetic modification for the stereospecific formation of a *m*-C_7N Manumycin core unit analogue. Additionally, the commercially available D-Tyrosine, having the opposite configuration on its tethered chiral chain, could also be used in future work as the starting material to produce the other enantiomer as the major product. Therefore, the production of either enantiomer form of the major isomer can now be controlled by the selection of the appropriate (D)- or (L)-Tyrosine starting material. Another benefit to using a spirolactone derivate is that acid hydrolysis of the minor product reverts back to the original starting material before oxidation. This would allow for an increase in the total conversion of the

starting material to the desired stereoisomer through the recycling of the minor product.

(3.3) Future Work

Future work associated with our findings would address the limitations currently existing in the thesis. In the synthesis of the racemic analogues, in Schemes 12 and 13, all but one of the reactions occurs with good yields. The epoxidation reaction has a low yield of 7 % and additional work needs to be done to increase the yield for this reaction. To solve this problem, further research could explore new reaction conditions with the current reagents to see if changing the reaction variables can increase the yield. Additional research could also focus on using new reagents and conditions for the epoxidation reaction in an effort to increase the yield while retaining the regio and stereochemistry of the current products. Supplemental analytical experimentation to confirm the syn configuration of the products using either an NOE experiment and/or x-ray crystallography should also be completed. The current core *m*-C_7N unit analogue products shown in Schemes 12 and 13, are only one reaction away from being actual *m*-C_7N core units. Therefore further research to achieve the cleavage of the lactone rings of the racemic products should also be continued and investigated.

The use of a tethered chiral chain on the (L)-Tyrosine derivatives in Scheme 14 allows for further experimentation regarding the diastereoselective

formation of compounds like (+)-**79** and **80**. This thesis was but a first attempt to show that using a chiral tethered chain could induce facial diastereoselectivity for the oxidative spiroannulation of spirolactone derivatives. Now that there is evidence to support our original assertions this opens up a large area to do further research for the many *para*-quinols compounds previously mentioned in Section 1.9 of the thesis. Initial research should examine the reaction mechanisms and the basis of diastereoselectivity caused by all three oxidants. The current low diastereoselectivity limits how effective the methodology is and further examination of the reaction mechanism should increase the likelihood of increasing the diastereoselectivity. The use of a chiral catalyst or reagent should also be considered as a means to improve the facial diastereoselective ratio. Concurrent research should also examine the implementation of new types of tethered chiral chains as a means to increase diastereoselectivity. By changing the sizes of the functional groups surrounding the chiral center, steric hindrance may also be enhanced with a possible corresponding increase in diastereomeric ratio. Additional research into oxidative spiroannulation would also entail using TTN to see if the different mechanism of the oxidant would amplify diastereoselectivity as Ponpipom *et al.*[109] had good success improving stereoselectivity using this reagent. Further investigation of the compounds (+)-**79** and **80** would entail a number of reactions consisting of a stereoselective epoxidation, a cleavage of the lactone ring and deamination of the sulfonylamide on the tethered chiral chain to produce manumycin analogues. In addition, further analytical experimentation to

confirm the syn configuration between the oxirane and the lactone oxygen using either an NOE experiment and/or x-ray crystallography.

(3.4) Final Remarks

The confirmation of our ability to induce diastereoselective facial control using a tethered chiral chain represents a small positive step towards our ultimate goal of producing compound **64** regio and stereoselectively. Additionally, this preliminary study demonstrates the first attempt at controlling facial selection for the formation of spirolactones and allows for the potential development of new synthetic pathways using spirolactones as a building block in the synthesis of natural products. These types of compounds, as has been previously mentioned, have exhibited numerous different biological activities and extensive biological testing is therefore recommended.

Chapter 4

Experimental

Infrared (IR) spectra were recorded on a FT-IR Perkins System 2000 spectrophotometer. Mass spectra were recorded with a Hewlett Packard (Agilent) 5989 B Mass spectrometer (MS) with a 5890 Series II Gas Chromatograph (GC). Optical rotations were obtained using a Rudolf Research Autopol III instrument. Flash column chromatographies were carried out using Silicycle silica gel (230-400 mesh, 60 Å). Analytical thin layer chromatography (tlc) was carried out on silica gel coated aluminum plates from Silicycle (60 Å, indicator F-254, thickness 250 μm). Visualization of tlc-plates was accomplished with UV light (Short-wave UV, 254 nm) and/or by staining with Vanillin (27g of vanillin, 50 mL water, 380 mL ethanol, 20 mL conc. sulfuric acid). ^{1}H (300.13 MHz) and ^{13}C (75.47 MHz) NMR spectra were recorded on a Bruker AMX 2-300 spectrometer using tetramethylsilane (TMS) as an internal calibration standard when using deuterated chloroform. When using other deuterated solvents, spectra were calibrated using chemical shifts of residue protons of the deuterated solvent used. Chemical shifts (δ) are quoted in parts per million (ppm) and ^{1}H spin coupling (J) values are in Hz. Two-dimensional NMR spectra were used to elicit further chemical shift information and confirm compound structures.

General Methodology of Small Scale Oxidations:

PIDA: To the starting material (20-30 mg, 1eq.) dissolved in acetone (10mL, 0 °C) was added PIDA in one portion (2.1 eq.) and the solution was stirred until completion. Reaction progress was followed by tlc (40-60 min). The solution was diluted with ethyl acetate (20-25 mL) and washed with cold water (10 mL). The organic fraction was dried ($MgSO_4$) and the solvent was evaporated. The residue was left under vacuum overnight to evaporate off phenyl iodine.

PIFA: To the starting material (20-30 mg, 1eq.) dissolved in acetone (10mL, 0 °C) was added PIFA in one portion (1.01 eq.) and the solution was stirred until completion. Reaction progress was followed by tlc (15-30 min.). The solution was diluted with ethyl acetate (20-25 mL) and washed with cold water (10 mL). The organic fraction was dried ($MgSO_4$) and the solvent was evaporated. The residue was left under vacuum overnight to evaporate off phenyl iodine.

LTA: To the starting material (20-30 mg, 1eq.) dissolved in acetone (10mL, 0 °C) was added LTA in one portion (3 eq.) and the solution was stirred until completion. Reaction progress was followed by tlc (15-30 min.). Ethylene glycol (4-5 drops) was added to the solution and it was left to stir overnight (14-16 hrs). The reaction mixture was filtered through Celite ® while rinsing with acetone (10-20 mL) and the solvent was evaporated. The residue was left under vacuum overnight to evaporate off ethylene glycol.

(2S)-2-{[(4-methlphenyl) sulfonyl] amino}-3-(4-{[(4-methylphenyl) sulfonyl] oxy} phenyl) propanoic acid **Intermediate to 65**: To a solution of L-Tyrosine (374 mg, 2.06 mmol, 1 eq.) in 1M NaOH (50 mL) was added a solution of TsCl (2.843 g, 14.91 mmol, 7 eq.) in diethyl ether (100 mL) in three portions (5 min. apart) at room temperature and the resulting mixture was stirred vigorously for 4-5 hrs. (confirmed by tlc: [2:8] MeOH/$CHCl_3$). The resulting white suspension was acidified with 10% HCl (pH 1) and extracted with ethyl acetate (3 x 100 mL). The organic fractions were combined, washed with saturated NaCl (150 mL) and dried ($MgSO_4$). The solvent was then evaporated to afford a white solid (crude 938 mg, 93 % yield). The product was used in the following reaction without further purification. Molecular Formula - $C_{23}H_{23}NO_7S_2$. Formula Weight - 489.56 g mole^{-1}. R_f (EtOAc) = .65. ^{1}H-NMR ($CDCl_3$) δ: 2.40 (s, 3H, H-7'), 2.44 (s, 3H, H-7"), 2.90 (m, 1H, H-3a), 3.08 (m, 1H, H-3b), 4.12 (m, 1H, H-2), 5.28 (d, 1H, J = 8.8, N-H), 6.81 (d, 2H, J = 8.3, H-5, H-9), 6.99 (d, 2H, J = 8.3, H-6, H-8) 7.21 (d, 2H, J = 8.1, H-2', H-6'), 7.31 (d, 2H, J = 8.1, H-2", H-6"), 7.55 (d, 2H, J = 8.1, H-3', H-5'), 7.67 (d, J = 8.1, H-3", H-5") 9.28 (broad s, 1H, CO_2H). ^{13}C-NMR ($CDCl_3$) δ: 21.74 (C- 7"), 21.94 (C- 7'), 38.24 (C- 3), 56.49 (C- 2), 122.69 (C- 5, C- 9), 127.20 (C- 3', C- 5') 128.66 (C- 3", C- 5"), 129.96 (C- 2', C- 6'), 130.04 (C- 2", C- 6") 130.87 (C- 6, C-8), 132.31 (C- 4), 134.17 (C- 4'), 136.31 (C- 4"), 144.32 (C- 1'), 145.75 (C- 1"), 148.99 (C- 7), 175.30 (C- 1).

(2S)-3-(4-hydroxylphenyl)-2-{[(4-methylphenyl) sulfonyl] amino} propanoic acid (**65**): To a solution of the **intermediate of 65** (750 mg, 1.534 mmol, 1 eq.) in ethanol (100 mL) was added a solution of 1M KOH (50 mL) and the white suspension was heated (77-82 ^{0} C) while stirring for 6-7 hrs (confirmed by tlc: [1:1] EtOAc/Hexane after mini work up, 10% HCl and extracting with EtOAc.). The resulting mixture was left to cool, then acidified with 10% HCl (pH~1) and extracted with ethyl acetate (150 mL, 50 mL). The organic fractions were combined, washed with saturated NaCl (150 mL) and dried ($MgSO_4$). The solvent was then evaporated to afford a white solid. This crude product was purified by column chromatography on silica gel, eluting with 40% ethyl acetate/hexane to afford an off white solid (477 mg, 92 % yield isolated). Molecular Formula – $C_{16}H_{17}NO_5S$. Formula Weight – 335.38 g mole^{-1}. R_f (EtOAc) = .10. ^{1}H-NMR (CD_3CN) δ: 2.39 (s, 3H, H-7'), 2.74 (m, 1H, H-3a), 2.92 (m, 1H, H-3b), 3.96 (m, 1H, H-2), 5.88 (d, 1H, J = 8.8, N-H), 6.63 (d, 2H, J = 8.4, H-5, H-9), 6.92 (d, 2H, J = 8.4, H-6, H-8) 7.25 (d, 2H, J = 8.1, H-2', H-6'), 7.52 (d, 2H, J = 8.3, H-3', H-5'). ^{13}C-NMR (CD_3CN) δ: 21.59 (C- 7'), 38.52 (C- 3), 58.27 (C- 2), 116.06 (C- 5, C-9), 127.77 (C- 3', C- 5'), 128.20 (C- 4), 130.54 (C- 2', C- 6'), 131.52 (C- 6, C-8), 138.47 (C- 4'), 144.58 (C- 1'), 156.90 (C- 7), 172.71 (C- 1).

N- [(3S) -1-oxaspiro [4.5] deca-6, 9-dien-2, 8-dion-3-yl]-4-methylbenzene sulfonamide (**66**): To a solution of **65** (177 mg, .528 mmol, 1 eq.) dissolved in acetone (15mL, 0 °C) was added PIFA (229 mg, .533 mmol, 1.01 eq.) in one

portion and the resulting mixture was stirred for 45-50 minutes (confirmed by tlc: [1:1] EtOAc/Hexane). The mixture was diluted with ethyl acetate (50 mL), then washed with cold water. The organic fraction was dried ($MgSO_4$) and the solvent evaporated to afford a Tan solid. The crude product was purified by column chromatography on silica gel, eluting with 50 % ethyl acetate/hexane to afford an off white solid (63 mg, 36 % yield isolated). Molecular Formula – $C_{16}H_{15}NO_5S$. Formula Weight – 333.359 g $mole^{-1}$. ^{1}H-NMR ($CDCl_3$) δ: 2.48(s, 3H, H-7'), 4.40 (m, 1H, H-2), 5.91 (d, 1H, J = 5.8, N-H), 6.26 (dd, 2H, J = 2, 10, H-5, H-9), 6.80 (dd, 2H, J = 2, 10, H-6, H-8), 7.33 (d, 2H, J = 8, H-3', H-5'), 7.80 (d, 2H, J = 8.2, H-2', H-6')

(2S)-3-(4-hydroxyl-3-nitrophenyl)-2-{[(4-methylphenyl) sulfonyl] amino} propanoic acid **67**: To a solution of 3-Nitro-L-Tyrosine (1.029 g, 4.55 mmol, 1 eq.) dissolved in 1M NaOH (100 mL) was added a solution of tetrahydrofuran (150 mL) with TsCl (7.012 g, 36.9 mmol, 9.5 eq.) in three portions (5-10 min. apart) and the resulting orange solution was stirred vigorously at room temperature. After 25-30 min. the solution turned a yellow colour, indicating an acidic environment (pH ~ 3), therefore more 1M NaOH (25 mL) was added. The solution then returned to an orange colour, which was left to stir overnight (14-16 hrs, confirmed by tlc ([2:8] MeOH/$CHCl_3$). The reaction mixture was acidified with 10% HCl (pH 1-2, orange to yellow colour change) and extracted with

dichloromethane (100mL, 50 mL). The organic fractions were combined, dried ($MgSO_4$) and the solvent evaporated to afford a yellow solid.

The crude yellow product was then dissolved in ethanol (100 mL) and 1M KOH (50mL) was added. The now orange reaction mixture was warmed (80-85 ^{o} C) and left to stir overnight (12-14 hrs. confirmed by tlc: [2:8] MeOH/$CHCl_3$, after mini work up, 10 % HCl and EtOAc). The resulting reaction mixture was cooled and then acidified with 10 % HCl (pH 1-2) causing the orange solution to turned yellow. Then concentrated the reaction mixture and yellow precipitates formed which were then extracted with dichloromethane (2 x 150 mL). The organic fractions were combined, dried ($MgSO_4$), and the solvent was then evaporated leaving a yellow solid. Recrystallization in benzene and drying under vacuum afforded a yellow solid product (1.485 g, 86 % yield). Molecular Formula – $C_{16}H_{16}N_2O_7S$. Formula Weight – 380.374 g $mole^{-1}$. Mpt: 137 ^{0}C. $[\alpha]_D$ = - 58.1 ^{0} (c: 0.155 g 100 mL^{-1} at 21 ^{0} C). FT-IR (KBR disk) cm^{-1}: 1734 (CO_2H), 1326, 1158 (SO_2NHR), 1539, 1430 (NO_2). ^{1}H-NMR ($CDCl_3$) δ: 2.40 (s, 3H, H-7'), 2.91 (m, 1H, H-3a), 3.15 (m, 1H, H-3b), 3.74 (broad s, 1H, OH), 4.16 (m, 1H, H-2), 5.42 (d, 1H, J = 8.5, N-H), 7.01 (d, 1H, J = 8.6, H-8), 7.20 (d, 2H, J = 8.3, H-2', H-6'), 7.38 (dd, 1H, J = 2.2, 8.6, H-9), 7.56 (d, 2H, J = 8.3, H-3', H-5'), 7.74 (d, 1H, J = 2.2, H-5), 10.46 (broad s, 1H, CO_2H). ^{13}C-NMR ($CDCl_3$) δ: 21.74 (C- 7'), 37.70 (C- 3), 56.59 (C- 2), 120.41 (C-8), 125.59 (C- 5), 127.18 (C- 3', C- 5'), 127.73 (C- 4), 129.91 (C- 2', C- 6'), 133.32 (C- 4'), 136.36 (C-7), 139.15 (C- 9), 144.39 (C- 1'), 154.50 (C- 6), 174.65 (C- 1).

(2E)-3-(4-hydroxyl-3-nitrophenyl) acrylic acid **69**:

To a solution of 4-hydroxyl-3-nitrobenzaldehyde (1.073 g, 6.43 mmol, 1 eq.) dissolved in pyridine (25 mL) was added piperidine (25 drops) and the resulting mixture was stirred (4-5 min.). Malonic acid (1.671 g, 16.1 mmol, 2.5 eq.) was then added in one portion and the resulting mixture was warmed (60-63 ^{o}C) and stirred overnight (12-14 hrs, confirmed by tlc: EtOAc, mini work up, 10 % HCl and EtOAc). The reaction was cooled and acidified (50 % HCl) until yellow precipitate formed (pH~2). This yellow precipitate was extracted with ethyl acetate (2 x 150 mL). The organic fractions were combined and washed with brine (150 mL), dried ($MgSO_4$), and the solvent was evaporated to afford a yellow solid. Removed excess solvent by vacuum and used without further purification (1.250 g, 93 % yield). Molecular Formula – $C_9H_7NO_5$. Formula Weight – 209.156 g mole^{-1}. FT-IR (KBR disk) cm^{-1}: 2942 (OH), 1684 (CO_2H), 1626 (C=C), 1533,1270 (NO_2). ^{1}H-NMR (Acetone-D_6) δ: 2.87 (broad s, 1H, OH), 6.58 (d, 1H, J= 16.0, H-2), 7.27 (d, 1H, J= 8.8, H-8), 7.70 (d, 1H, J= 16.4, H-3), 8.08 (d, 1H, J= 2.2, 8.5, H-9), 8.40 (d, 1H, J = 2.2, H-5), 10.67 (broad s, 1H, CO_2H). The ^{13}C-NMR of this compound agrees with the previously published data.[142]

3-(3-amino-4-hydroxylphenyl) propanoic acid **70**:

Detail procedure for formation of **70** can be found in experimental procedure of **71** and **72** below. Initial attempts to purify by recrystallization and

column chromatography failed due to reactive qualities of product therefore used filtered solution of **70** directly for producing both amides **71** and **72**. A small aliquot was used to acquire a ^{1}H-NMR of the crude product. Molecular Formula – $C_9H_{11}NO_3$. Formula Weight – 181.189 g mole^{-1}. ^{1}H-NMR (D_3C-OD) δ: 2.46 (t, 2H, J = 7.0, H-3), 2.69 (t, 2H, J = 7.9, H-2), 6.48 (m, 1H, H-5), 6.60 (m, 2H, H-8, H-9),

3-[3-(acetylamino)-4-hydroxylphenyl] propanoic acid **71**:

To a solution of **69** (210 mg, 1.00 mmol, 1eq.) dissolved in THF (20 mL) was added the catalyst 10 % palladium-on-charcoal (15 % by mass, 32 mg). The resulting mixture was then placed on a hydrogenator, flushed (5 times) with hydrogen, and left to agitate under pressure (39 psi.) for 6-7 hrs. The reaction mixture was vented and then vacuumed filtered through Celite ® rinsing with THF (25-30 mL). AcCl (79 mg, 1.13 mmol, 1.13 eq.) was directly added to the filtered solution containing **70** and left to stir at room temperature for 60 min. Water was added (15 mL) and extracted with EtOAc (2 x 50 mL). The organic fractions were combined and washed with saturated NaCl (50mL), dried ($MgSO_4$), and the solvent was evaporated off. The product was re-crystallized with Hexane/Acetone to afford a white solid (104 mg), with a 47 % yield from compound **69**. Molecular Formula – $C_{11}H_{13}NO_4$. Formula Weight – 223.225 g mole^{-1}. FT-IR (KBR disk) cm^{-1}: 3393 (NH, OH), 1699 (CO_2H), 1657 (NHAc). ^{1}H-NMR (CD_3CN) δ: 2.15 (s, 3H, H-2'), 2.54 (t, 2H, J = 7.5, H-3), 2.78 (t, 2H, J = 7.5, H-2), 6.82 (d, 1H, J = 8.3,

H-8), 6.95 (dd, 1H, J = 2.1, 8.3, H-9), 7.06 (d, 1H, J = 2.0, H-5), 8.53 (broad s, 1H, OH), 8.81 (s, 1H, NH). ^{13}C-NMR (CD_3CN) δ: 23.53 (C- 2'), 30.96 (C- 3), 35.96 (C- 2), 119.50 (C-5), 123.04 (C- 8), 127.10 (C- 6), 127.31 (C- 9), 133.69 (C- 4), 147.89 (C- 7), 172.09 (C-1), 174.42 (C-1').

3-[3-(benzoylamino)-4-hydroxylphenyl] propanoic acid **72**:

To a solution of **69** (222 mg, 1.06 mmol, 1eq.) dissolved in THF (20 mL) was added the catalyst 10 % palladium-on-charcoal (15 % by mass, 33 mg). The resulting mixture was then placed on a hydrogenator, flushed (5 times) with hydrogen and left to agitate under pressure (36 psi.) overnight (12 hrs) while recharging hydrogen pressure twice (36 psi.) until hydrogen up-take by reaction mixture stopped (pressure did not decrease for 1-2 hrs.). The reaction mixture was vacuum filtered through Celite ® rinsing with THF. To the filtered solution containing **70** was directly added BzCl (154 mg, 1.1 mmol, 1 eq.) and left to stir at room temperature for 30 min. Then 10 % HCl (25 mL) was added and stirring continued an additional 5 min. followed by extraction with CH_2Cl_2 (2 x 35 mL). The organic fractions were combined, dried ($MgSO_4$), and evaporated off solvent. The resulting mixture was re-crystallized with Hexane/Acetone to afford an off white solid (250 mg) with an 83 % yield from compound **69**. Molecular Formula – $C_{16}H_{15}NO_4$. Formula Weight – 285.295 g mole^{-1}. FT-IR (KBR disk) cm^{-1}: 3201 (NH, OH), 1692 (CO_2H), 1636 (NHAc). ^{1}H-NMR (Acetone-D_6) δ: 2.60 (t, 2H, J = 7.4, H-3), 2.84 (t, 2H, J = 7.9, H-2), 6.89 (d, 1H, J = 8.2, H-8), 7.00 (dd, 1H, J =

2.1, 8.25, H-9), 7.57 (m, 4H, H-5, H-4', H-5', H-6'), 8.05 (d, 2H, J = 8.2, H-3', H-7'), 9.07 (broad s, 1H, NH), 9.54 (broad s, 1H, OH), 10.58 (broad s, 1H, CO_2H). ^{13}C-NMR (Acetone-D_6) δ: 30.87 (C- 3), 36.21 (C- 2), 118.69 (C- 8), 123.31 (C-5), 123.41 (C- 6), 126.88 (C- 9), 127.37 (C- 4), 128.54 (C-4', C-6'), 129.61 (C-3', C-7'), 132.99 (C-5'), 134.99 (C-2'), 148.03 (C-7), 167.34 (C-1'), 173.94 (C-1).

N-(1-oxaspiro[4.5]deca-6,9-dien-2,8-dion-7-yl)acetamide **(+/-)-73:**

To a solution of **71** (122 mg, .547 mmol, 1 eq.) dissolved in acetone (10 mL, 0 °C) was added PIFA (306 mg, .711 mmol, 1.3 eq.) in one portion and stirred for 20-25 minutes (confirmed by tlc: [1:1] EtOAc/Hexane). The reaction mixture was diluted with ethyl acetate (15 mL), washed with cold water (10 mL), dried organic fraction ($MgSO_4$) and evaporated off solvent to afford a Tan solid. The crude product was purified by re-dissolving with $CHCl_3$, filtering of the solution through Celite ®, evaporating off the solvent and placing it under vacuum overnight to afford an off white solid (120 mg, 98 % yield). Molecular Formula – $C_{11}H_{11}NO_4$. Formula Weight – 221.209 g mole^{-1}. FT-IR (KBR disk) cm^{-1}: 3333 (NH), 1777 (lactone), 1668 (amide), 1650 (ketone), 1620 (α, β-conjugation to ketone). ^{1}H-NMR ($CDCl_3$) δ: 2.17 (s, 3H, H-2'), 2.44 (m, 2H, H-4), 2.81 (m, 2H, H-3), 6.35 (d, 1H, J = 10.0, H-9), 6.94 (dd, 1H, J = 3.1, 10.0, H-10), 7.75 (d, 1H, J = 3.1, H-6), 7.99 (broad s, 1H, NH). ^{13}C-NMR ($CDCl_3$) δ: 24.86 (C- 2'), 28.36 (C- 4), 32.91 (C- 3), 79.76 (C-5), 124.30 (C- 6), 127.12 (C- 9), 131.55 (C- 7), 148.37 (C-10), 169.51 (C-1'), 175.46 (C-2), 179.40 (C-8).

N-(1-oxaspiro[4.5]deca-6,9-dien-2,8-dion-7-yl)benzamide **(+/-)-74**:

To a solution of **71** (262 mg, .92 mmol, 1 eq.) dissolved in acetone (30 mL, 0 °C) was added PIFA (396 mg, .92 mmol, 1 eq.) in one portion and stirred for 20-25 minutes (confirmed by tlc: [1:1] EtOAc/Hexane). The reaction mixture was diluted with ethyl acetate (100 mL), washed with cold water (50 mL), dried organic fraction ($MgSO_4$) and the solvent was evaporated to afford a Tan solid. The crude product was purified by column chromatography on silica gel, eluting with (40) % ethyl acetate/hexane to afford an off white solid (243 mg, 86 % yield isolated). Molecular Formula – $C_{16}H_{13}NO_4$. Formula Weight – 283.279 g $mole^{-1}$. FT-IR (KBR disk) cm^{-1}: 3381 (NH), 1781 (lactone), 1665 (amide), 1650 (ketone), 1621 (α, β-conjugation to ketone). ^{1}H-NMR ($CDCl_3$) δ: 2.49 (m, 2H, H-4), 2.84 (m, 2H, H-3), 6.42 (d, 1H, J = 10.0, H-9), 6.99 (dd, 1H, J = 3.1, 10.0, H-10), 7.54 (m, 3H, H-4', H-5', H-6'), 7.86 (m, 2H, H-3', H-7'), 7.95 (d, 1H, J = 3.1, H-6), 8.81 (broad s, 1H, NH). ^{13}C-NMR ($CDCl_3$) δ: 28.40 (C- 4), 33.02 (C- 3), 79.84 (C- 5), 124.54 (C-6), 127.20 (C- 9), 127.32 (C- 4', C- 6'), 129.16 (C- 3', C- 7'), 131.74 (C-7), 132.72 (C-5'), 133.87 (C-2'), 148.60 (C-10), 166.21 (C-1'), 175.44 (C-2).

N- [(1'S, 2R, 6'R)-5,5'-dioxo-4, 5-dihydro-3H-spiro [furan-2, 2'-[7] oxabicyclo [4.1.0] hept [3] en]-4'-yl] benzamide **76**: To a solution of (+/-)-**74** (55mg, .14 mmol, 1 eq.) dissolved in 3:1 THF/H_2O (4 mL, 0 °C) was added ~ 30 % H_2O_2 (200 μL, 67 mg, 2 mmol, 14 eq.) and stirred (6 hrs, confirmed by tlc: [1:1]

EtOAc/Hexane). The reaction mixture was diluted with water (15 mL) and extracted with ethyl acetate (2 x 25 mL). The organic fractions were combined, dried ($MgSO_4$), and evaporated solvent to afford a solid. The crude product was purified by column chromatography on silica gel, eluting with 50 % ethyl acetate/hexane to afford an off white solid. The resulting mixture was triturated with HPLC hexane to remove some impurity (3 mg, 7 % yield). Molecular Formula – $C_{16}H_{13}NO_5$. Formula Weight – 299.278 g $mole^{-1}$. ^{1}H-NMR ($CDCl_3$) δ: 2.48 (t, 2H, J = 8.4, H-3), 2.85 (m, 2H, H-4), 3.72 (d, 1H, J = 4, H-7), 3.77 (m, 1H, H-6), 7.54 (m, 3H, H-4', H-5', H-6'), 7.72 (d, 1H, J = 2.7, H-10), 8.35 (broad s, 1H, NH). ^{13}C-NMR ($CDCl_3$) δ: 27.92 (C- 4), 33.02 (C- 3), 51.47 (C- 6), 55.37 (C-7), 81.54 (C- 5), 123.89 (C- 9), 127.26 (C- 4', C- 6'), 129.01 (C- 3', C- 7'), 129.20 (C-10), 132.81 (C-5'), 133.75 (C-2'), 166.28 (C-1'), 174.86 (C-2), 188.18 (C-8).

(2S)-3-(3-amino-4-hydroxylphenyl)-2-{[(4-methylphenyl) sulfonyl] amino} propanoic acid **77**: Initial attempts to purify by recrystallization and column chromatography failed due to reactive qualities of product therefore used filtered solution directly for producing the amide **78**. A small reaction was repeated to acquire a ^{1}H-NMR for analytical information of the crude product (see experimental procedure of **78** below). Molecular Formula – $C_{16}H_{18}N_2O_5S$. Formula Weight – 350.391 g $mole^{-1}$. ^{1}H-NMR (D_2O + Na_2CO_3) with a trace of EtOH solvent, δ: 2.35 (s, 3H, H-7'), 2.45 (m, 1H, H-3a), 2.78 (m, 1H, H-3b), 3.59

(m, 1H, H-2, EtOH), 6.33 (m, 3H, H-5, H-8, H-9), 7.22 (d, 2H, J = 8.6, H-3', H-5'), 7.39 (d, 2H, J = 8.3, H-2', H-6').

(2S)-3-[3(acetylamino)-4-hydoxylphenyl]-2-{[(4-methylphenyl) sulfonyl] amino} propanoic acid **78**: To a solution of **67** (166 mg, .437 mmol, 1eq.) dissolved in THF (25 mL) was added the catalyst 10 % palladium-on-charcoal (15 % by mass, 26 mg). The resulting mixture was then placed on a hydrogenator, flushed (5 times) with hydrogen and left to agitate under pressure (39 psi.) for 15-16 hrs. The reaction mixture was vented and then vacuumed filtered through Celite ® rinsing with THF (25-30 mL). To the filtered solution containing **77** was directly added AcCl (51 mg, .66 mmol, 1.5 eq.) and left to stir at room temperature overnight. The reaction mixture was diluted with CH_2Cl_2 (50 mL), washed with saturated NaCl (15 mL), dried ($MgSO_4$) and evaporated off solvent. It was then re-crystallized with Hexane/Acetone to afford a white solid (68 mg), with a 40 % yield from compound **67**. Molecular Formula – $C_{18}H_{20}N_2O_6S$. Formula Weight – 392.427 g mole^{-1}. $[\alpha]_D$ = - 88.9 ^{0} (c: 0.018 g 100 mL^{-1} at 21 ^{0} C). FT-IR (KBR disk) cm^{-1}: 3257 (NH), 1777 (CO_2H), 1657 (amide), 1289, 1157 (SO_2). ^{1}H-NMR (Acetone-D_6) and a trace of EtOAc solvent, δ: 2.20 (s, 3H, H-2"), 2.38 (s, 3H, H-7'), 2.83 (m, 1H, H-3a), 2.93 (m, 1H, H-3b), 4.04 (m, 1H, H-2, EtOAc), 6.64 (d, 1H, NH-2), 6.70 (d, 1H, J = 8.2, H-8), 6.83 (dd, 1H, J = 2.1, 8.2, H-9), 7.15 (broad s, 1H, H-5), 7.25 (d, 2H, J = 7.9, H-2', H-6'), 7.55 (d, 2H, J = 8.3, H-3', H-5'), 9.25 (broad s, 1H, CO_2H). ^{13}C-NMR (Acetone-D_6) δ: 21.48 (C- 2"), 23.50 (C- 7'), 37.70

(C- 3), 58.29 (C- 2), 118.97 (C-5), 123.04 (C- 8), 127.30 (C- 6), 127.73 (C- 3', C- 5'), 127.87 (C- 9), 128.72 (C- 4'), 130.25 (C- 4, C- 2', C- 6'), 139.11 (C- 1''), 143.80 (C-1'), 148.34 (C- 7), 172.61 (C- 1).

N- ((3S)-3-{[(4-methylphenyl) sulfonyl] amino}-1-oxaspiro [4.5] deca-6, 9-dion-2, 8-dien-7-yl) acetamide (+)-**79**: To a solution of **78** (118 mg, .30 mmol, 1 eq.) dissolved in acetone (20 mL, 0 ^{0}C) was added PIFA (142 mg, .331 mmol, 1.1 eq.) in one portion. After confirming reaction completion by tlc (25 min.), the reaction mixture was diluted with EtOAc (25 mL), washed with cold water (15 mL), dried solvent ($MgSO_4$) and evaporated off solvent. The crude product was purified by column chromatography on silica gel, eluting with 50 % ethyl acetate/hexane to afford the major and minor diastereomer as white solids. The resulting mixture was triturated with HPLC hexane to remove most of the impurity. Isolated 38 mg of the major diastereomer, 54 mg of a mixture of the major and minor diastereomer and 8 mg of the minor diastereomer (100 mg total, 85 % total yield). Analytical information is reported for major diastereomer plus **79**. Molecular Formula – $C_{18}H_{18}N_2O_6S$. Formula Weight – 390.410 g mole^{-1}. $[\alpha]_D$ = + 55.6 ^{0} (c: 0.036 g 100 mL^{-1} at 22 ^{0} C). FT-IR (KBR disk) cm^{-1}: 3327 (NH), 1778 (lactone), 1654 (amide), 1645 (ketone), 1631 (α, β-conjugation to ketone), 1339, 1161 (SO_2). ^{1}H-NMR (CD_3CN) trace of acetone solvent, δ: 2.16 (s, 3H, H-7'), 2.28 (m, 1H, H-4a), 2.45(s, 3H, H-2''), 2.46 (m, 1H, H-4b), 3.54 (m, 1H, H-3), 6.12 (d, 1H, J = 8.0, NH-2), 6.25 (d, 1H, J = 10.0, H-9), 7.06 (dd, 1H, J = 3.1, 10.0, H-10), 7.38 (d, 2H,

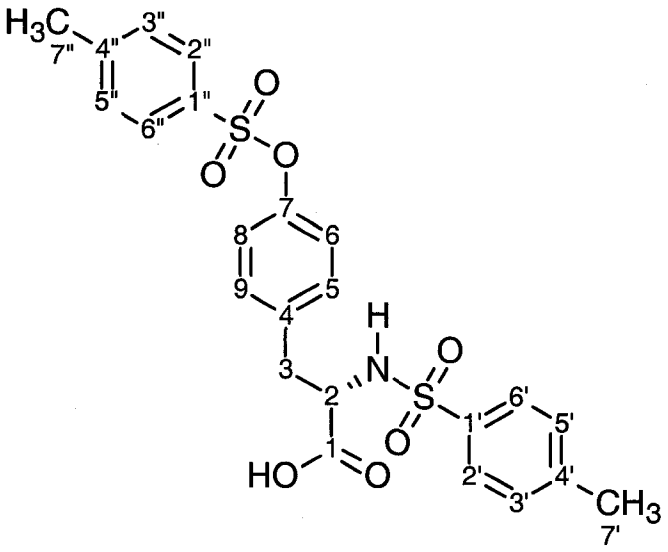
J = 8.0, H-2', H-6'), 7.52 (d, 1H, J = 3.1, H-6), 7.78 (d, 2H, J = 8.3, H-3', H-5'), 8.18 (broad s, 1H, NH-7). ^{13}C-NMR (CD_3CN) δ: 21.59 (C- 2"), 24.72 (C- 7'), 39.96 (C- 4), 52.97 (C- 3), 78.42 (C-5), 124.78 (C- 6), 127.48 (C- 9), 127.95 (C-3', C- 5'), 130.84 (C- 2', C- 6'), 133.51 (C- 7), 138.81 (C- 4'), 145.16 (C-1'), 147.41 (C- 10), 170.93 (C- 1"), 173.63 (C- 2).

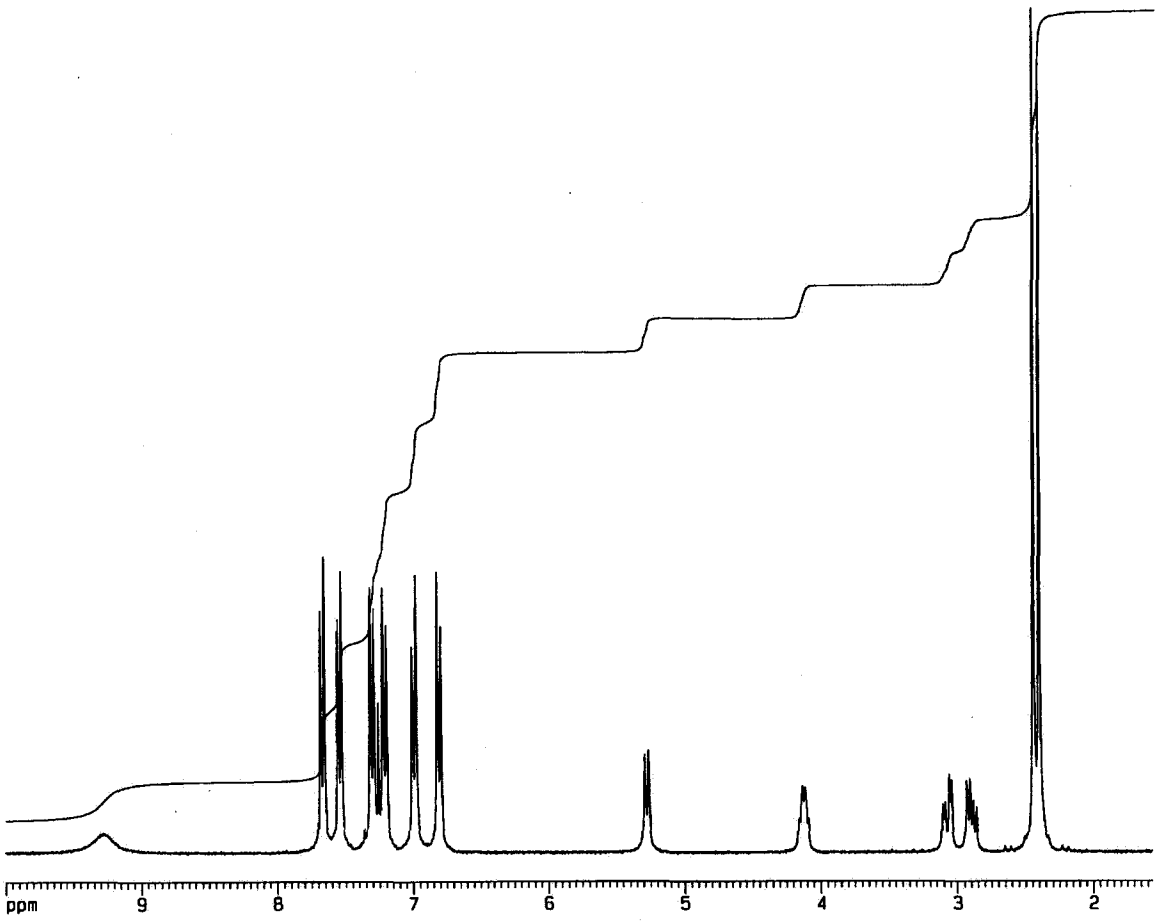
NMR Spectra of Compounds in Numerical Order

(All values are in ppm)

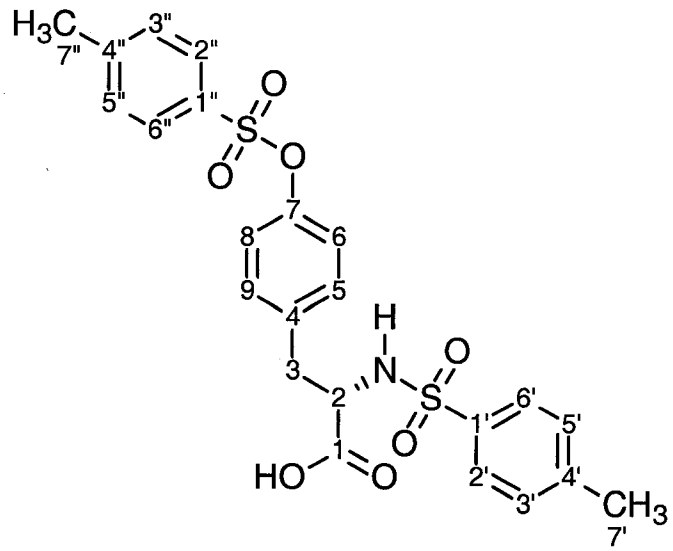
Appendix 1

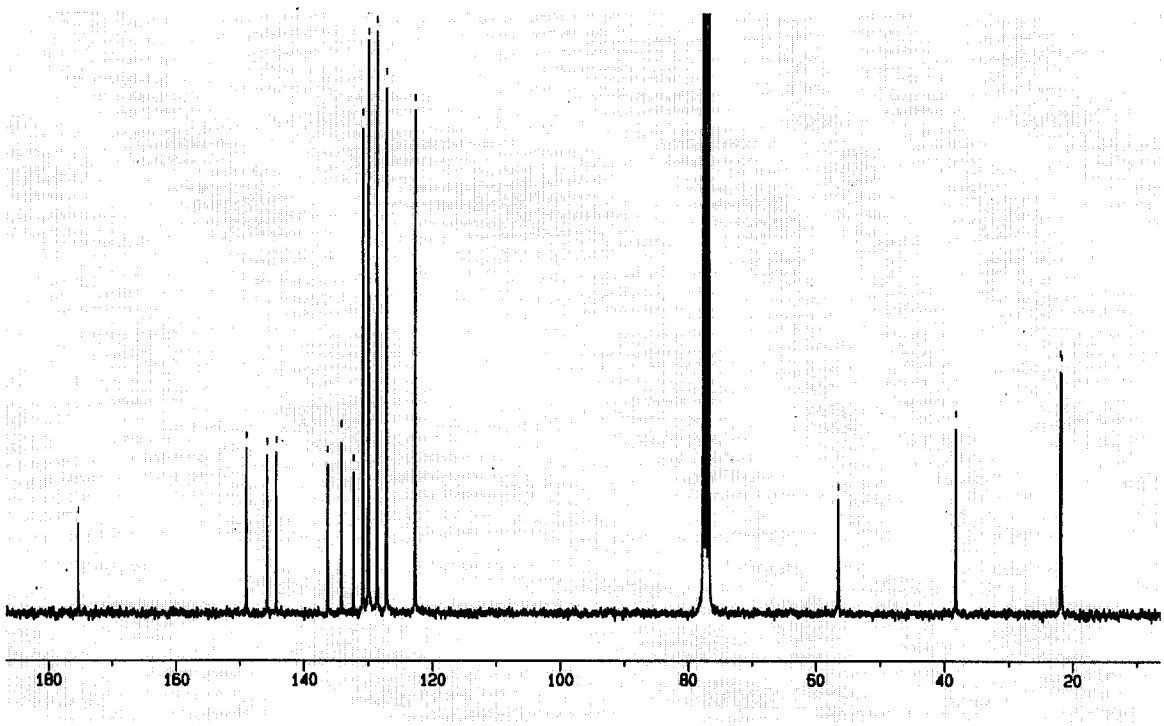
^{1}H-NMR Spectrum of Intermediate to Compound **65**



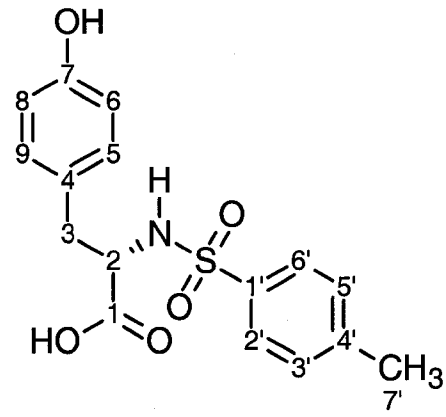


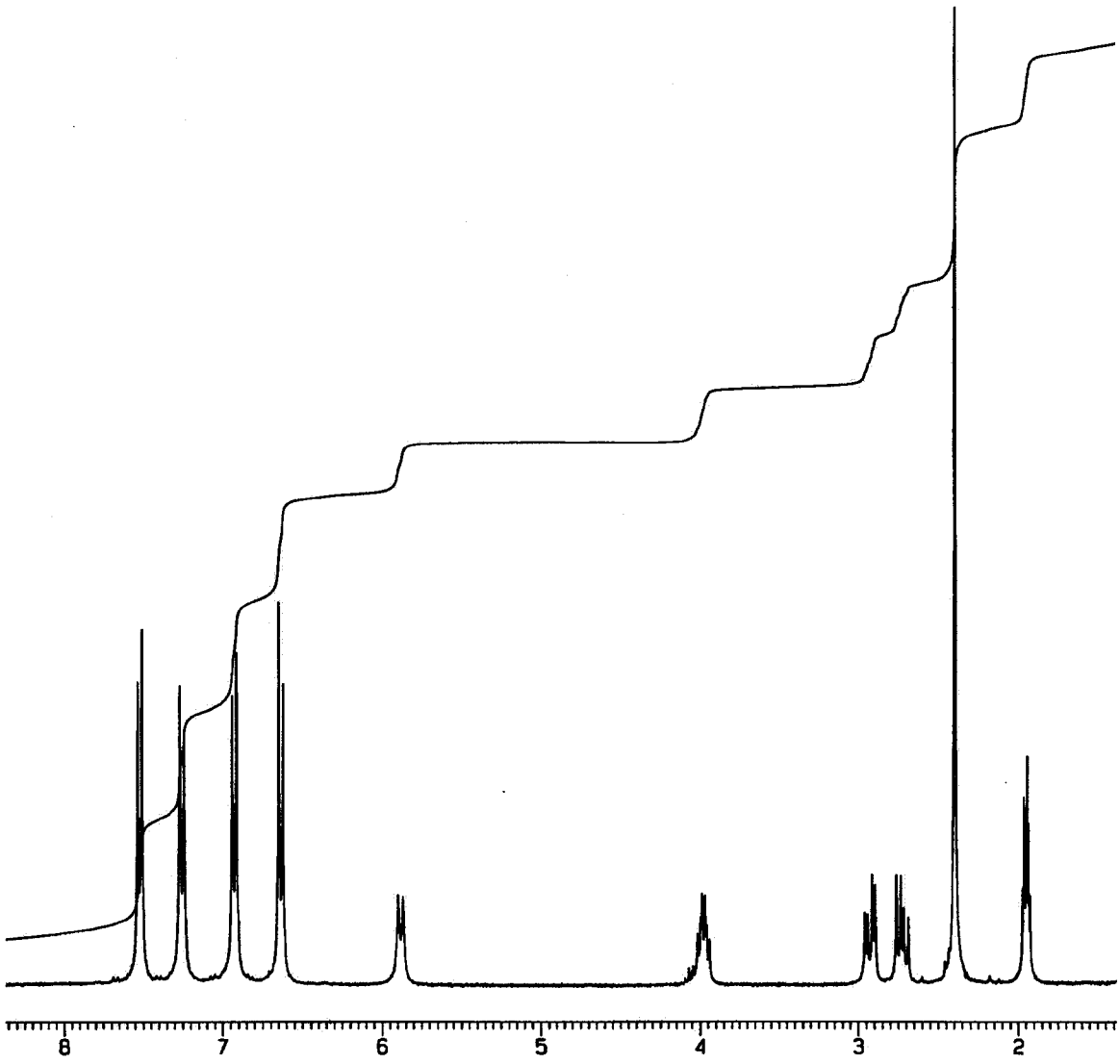
^{13}C-NMR Spectrum of Intermediate to Compound **65**



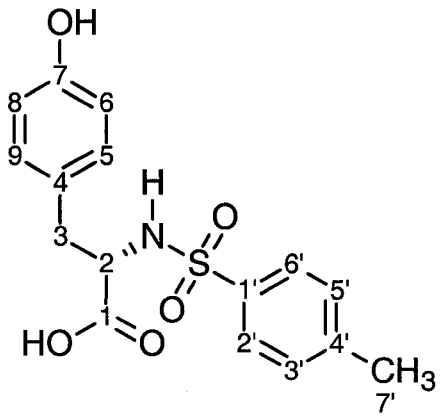


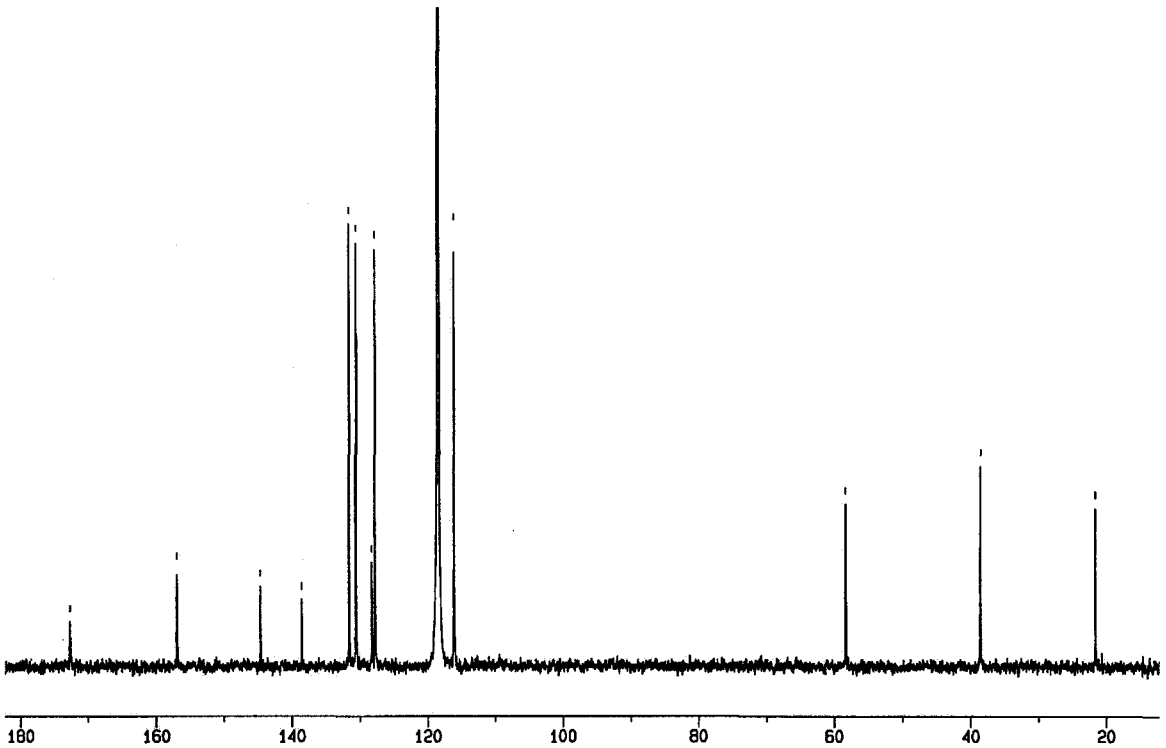
^{1}H-NMR Spectrum of Compound **65**



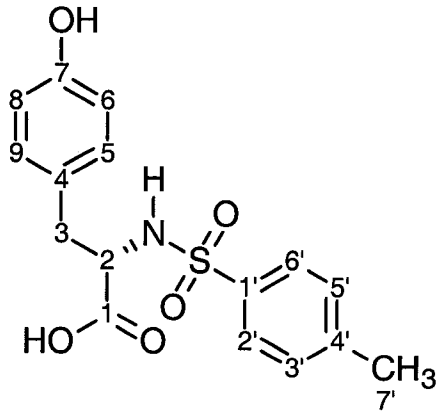


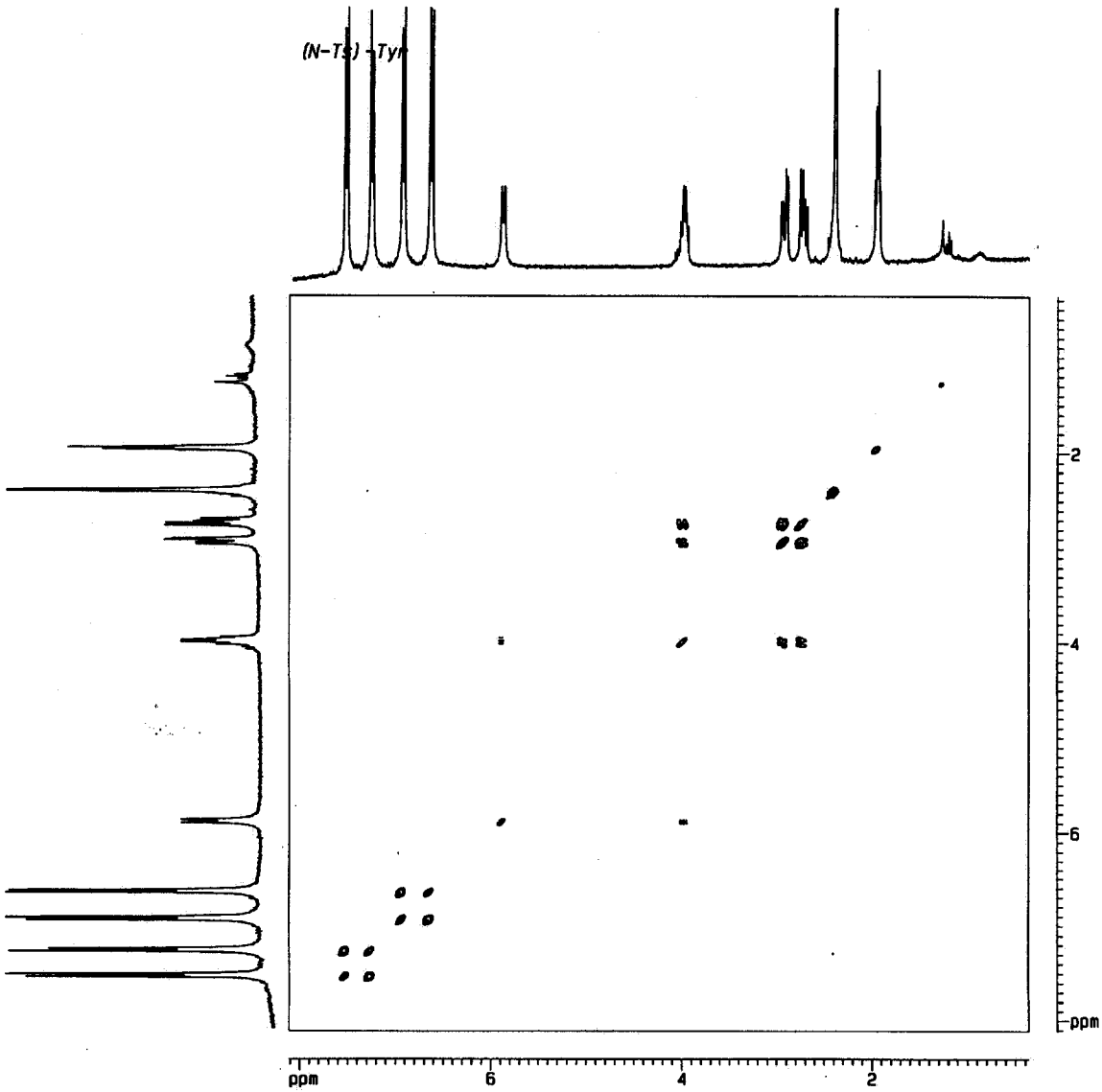
^{13}C-NMR Spectrum of Compound **65**



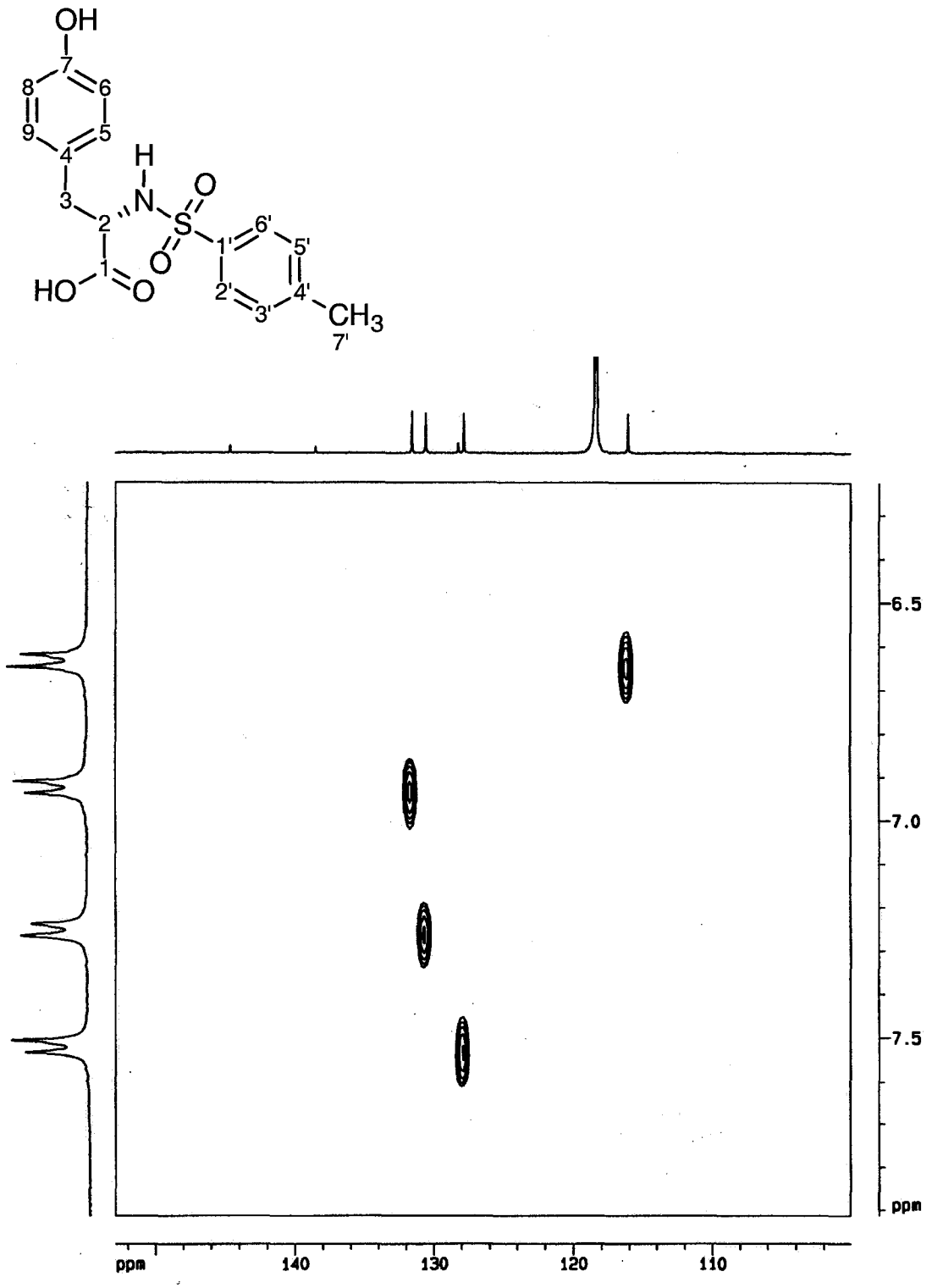


COSY-NMR Spectrum of Compound **65**

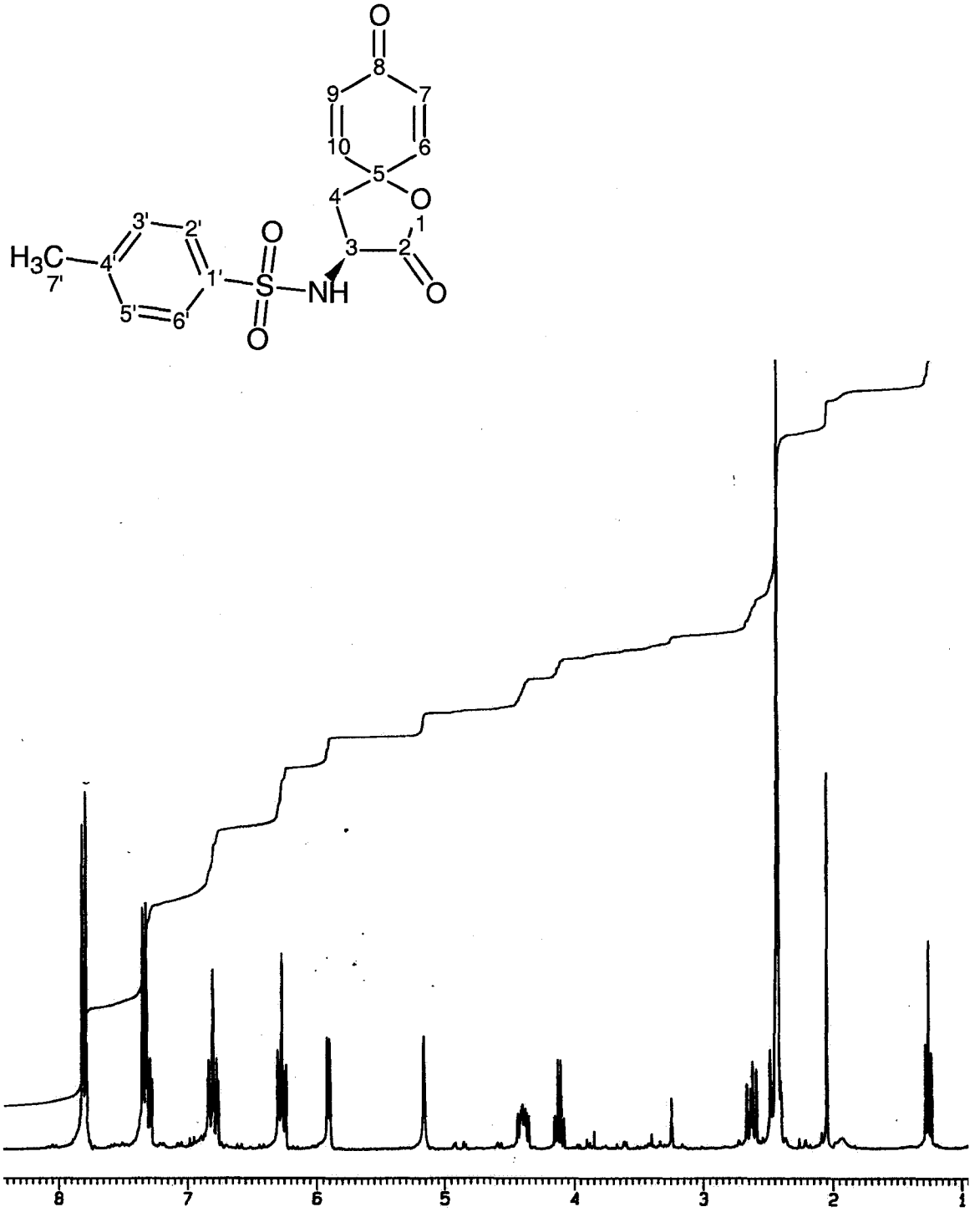




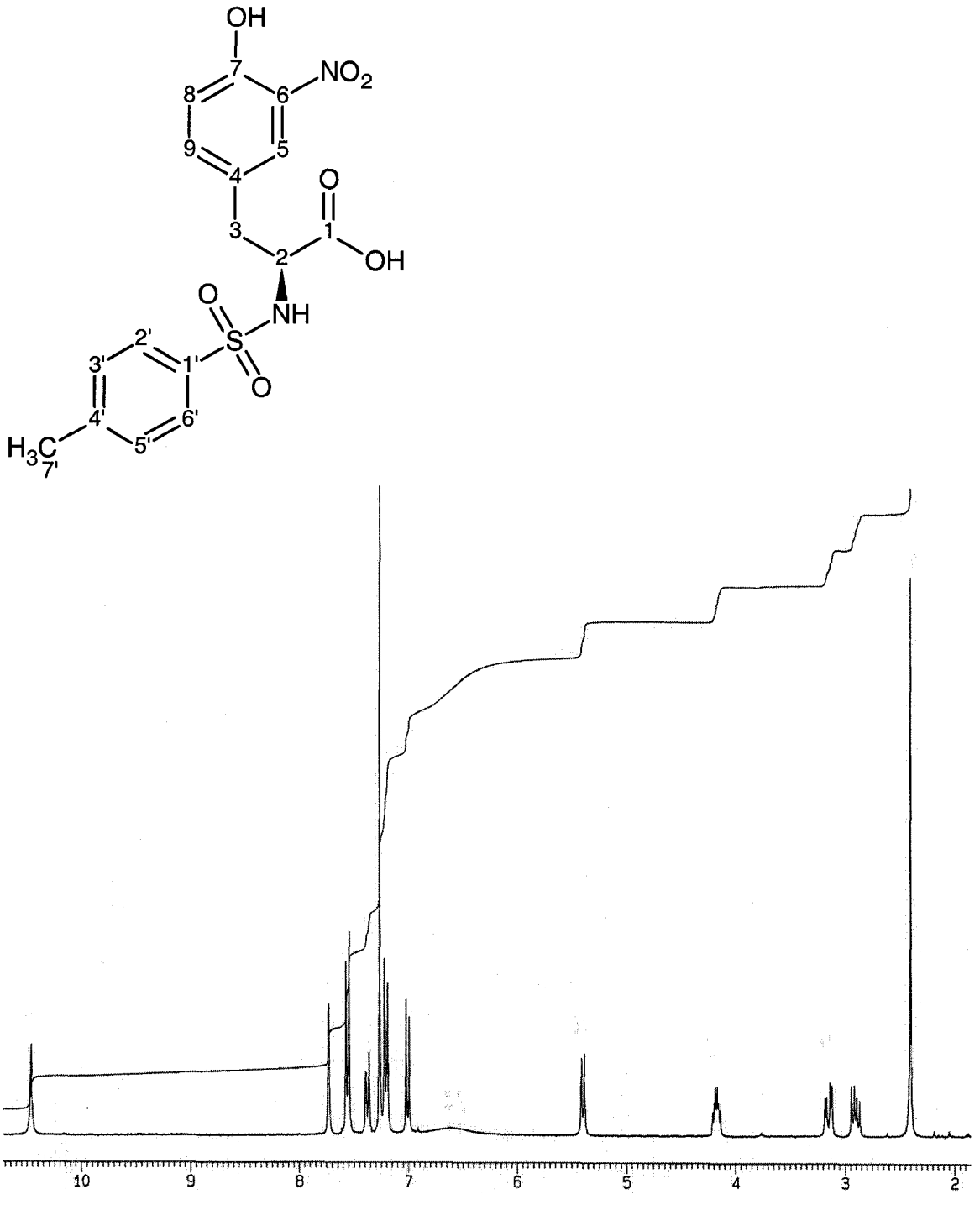
HETCOR-NMR Spectrum of Compound **65**



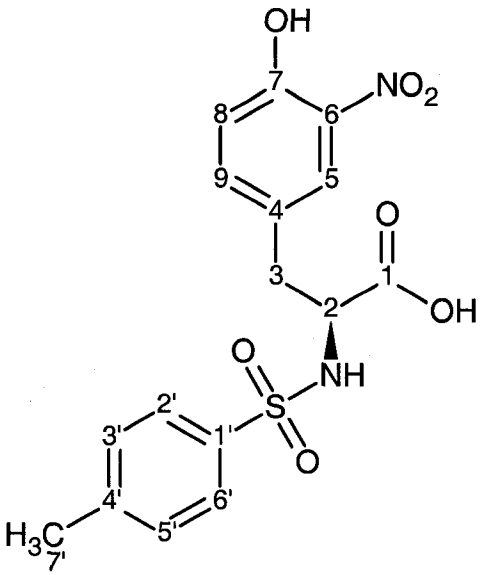
[1]H-NMR Spectrum of Compound **66**

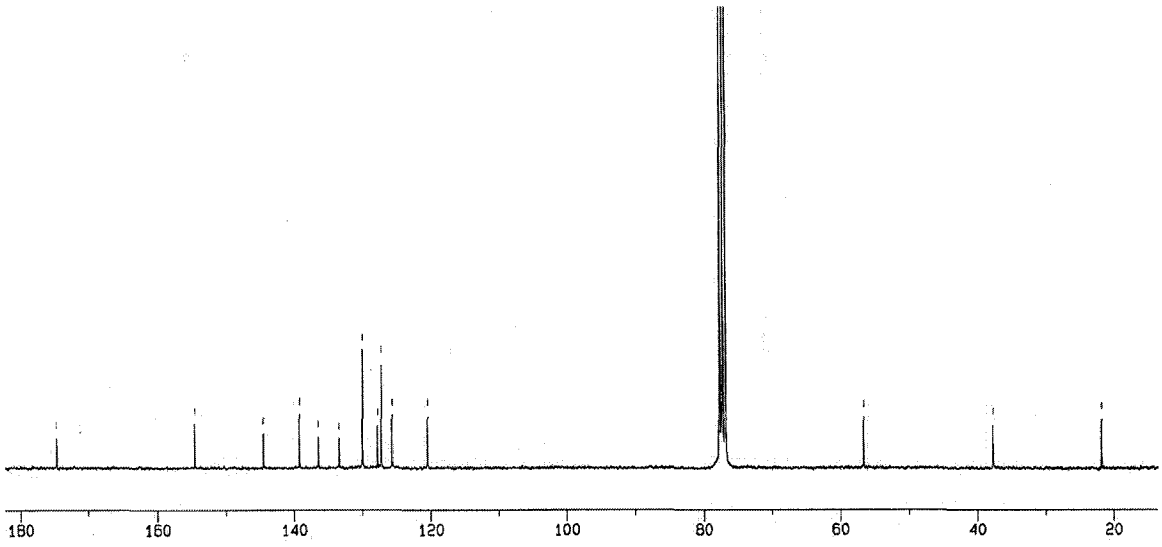


^{1}H-NMR Spectrum of Compound **67**

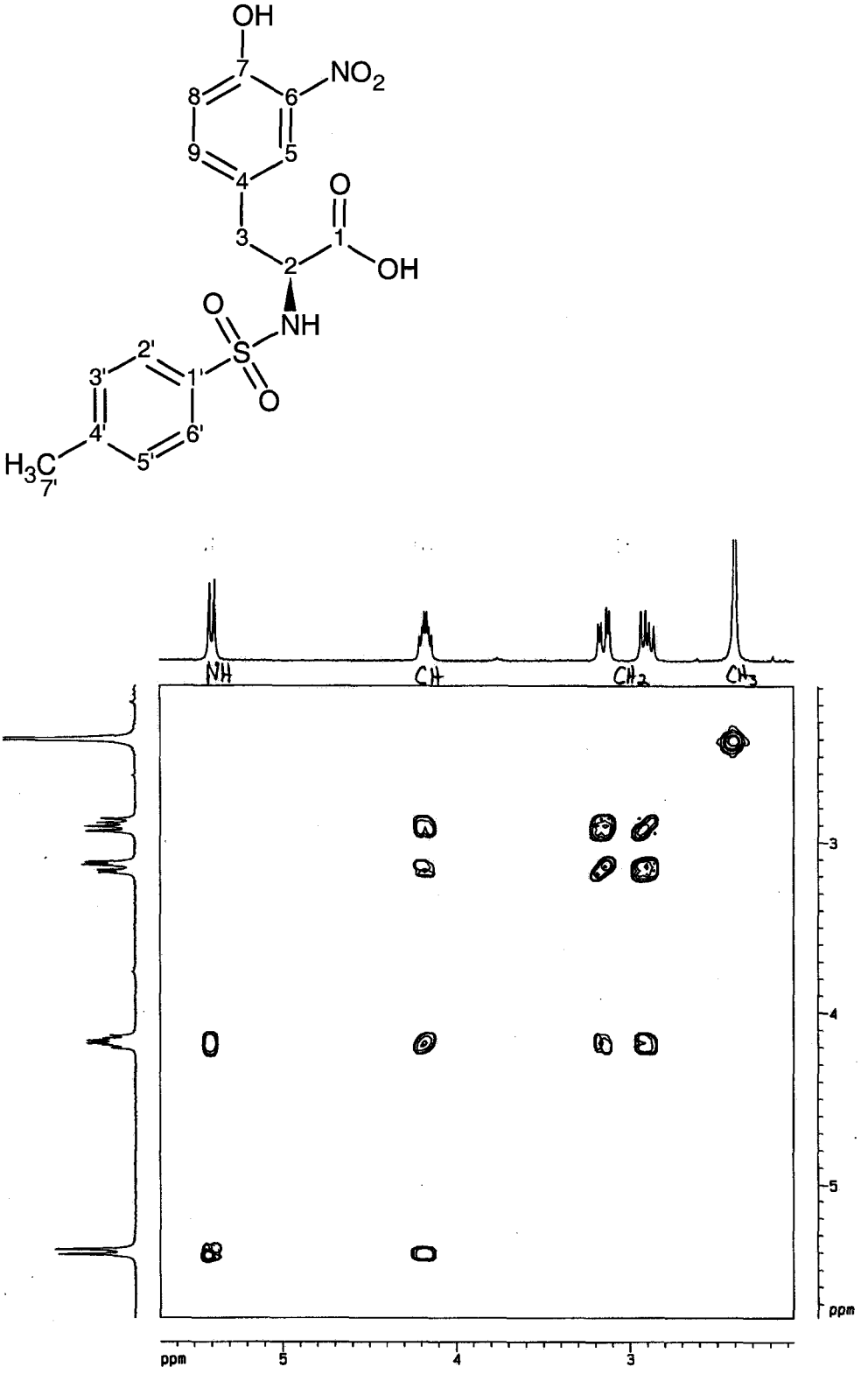


^{13}C-NMR Spectrum of Compound **67**

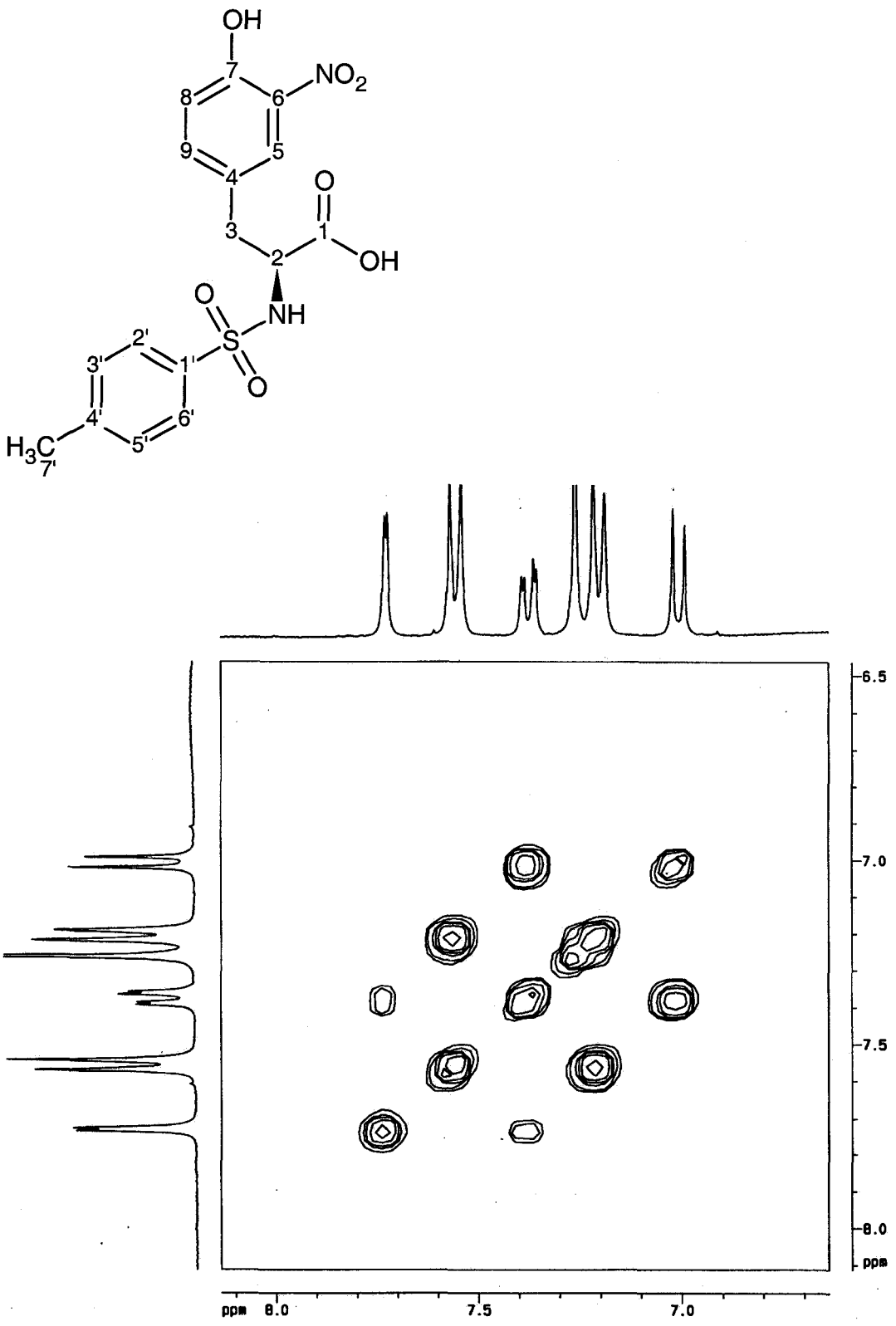




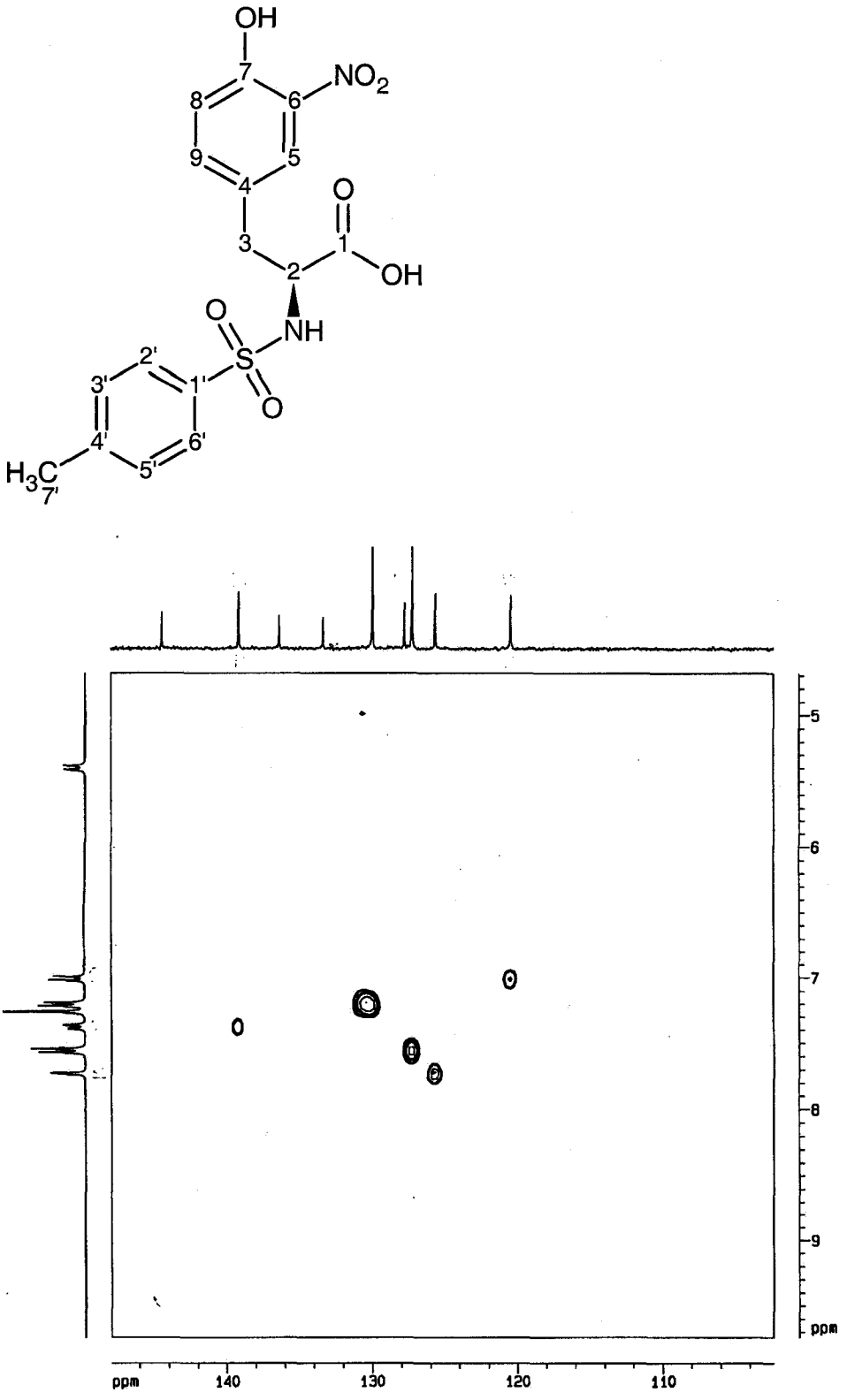
COSY-NMR Spectrum of Compound **67**



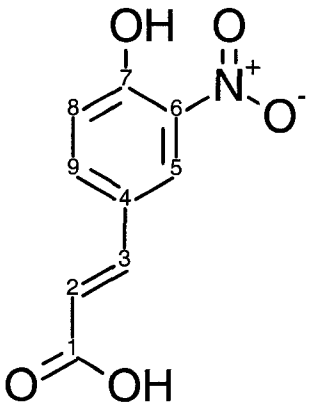
COSY-NMR Spectrum of Compound **67**

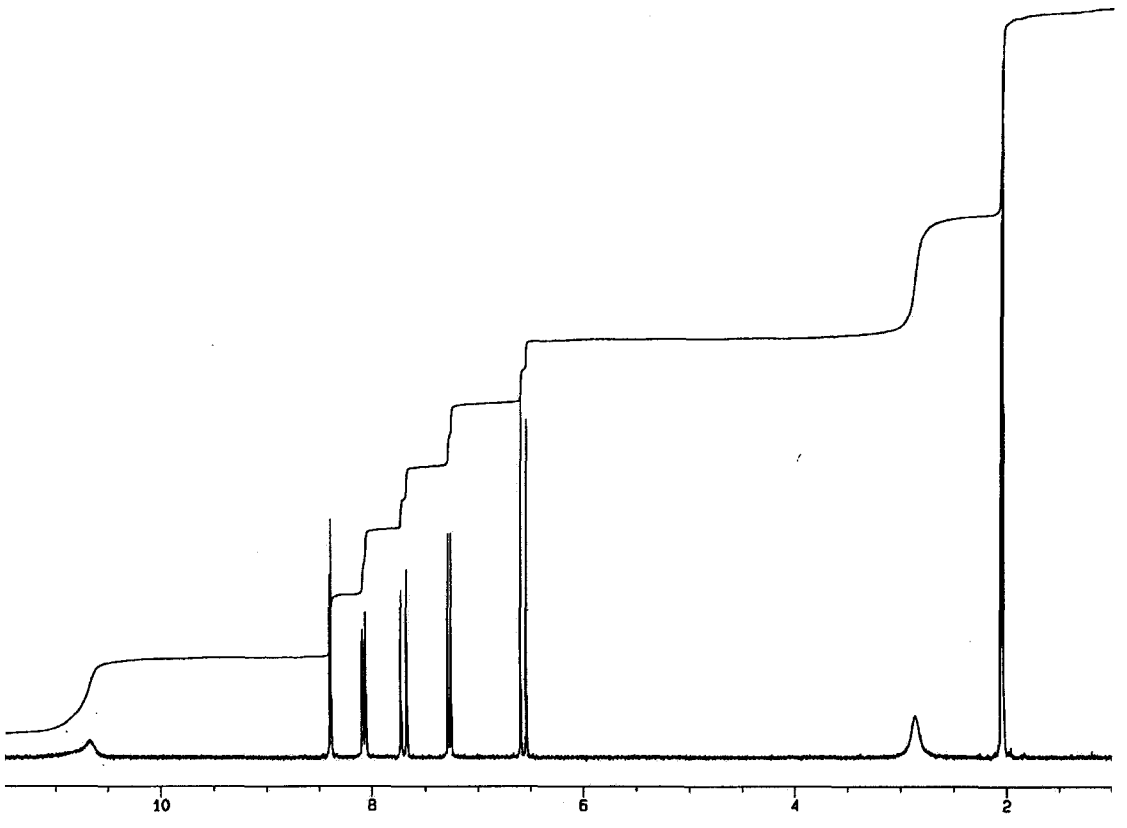


HETCOR-NMR Spectrum of Compound **67**

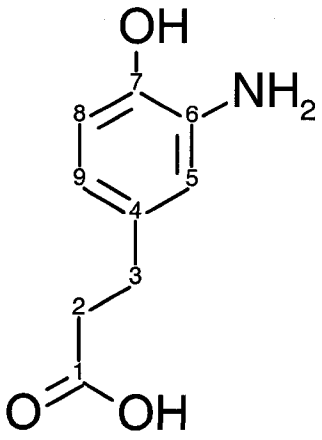


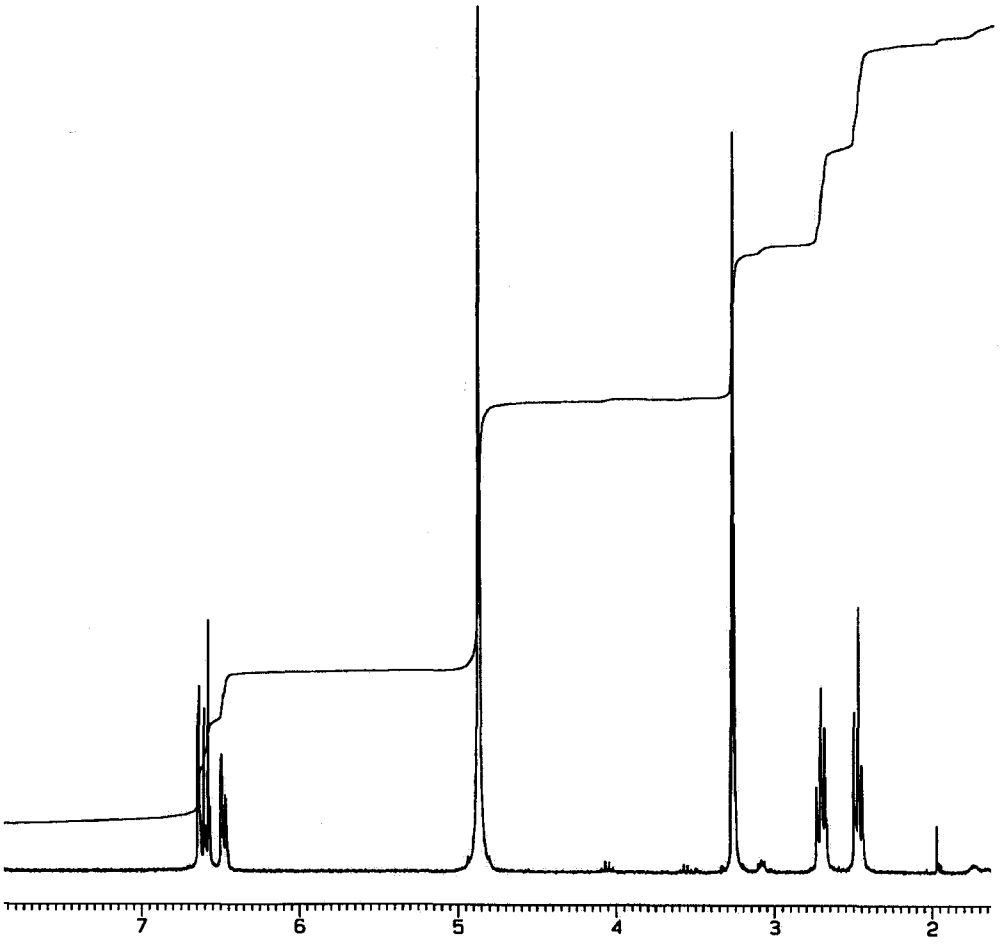
^{1}H-NMR Spectrum of Compound **69**



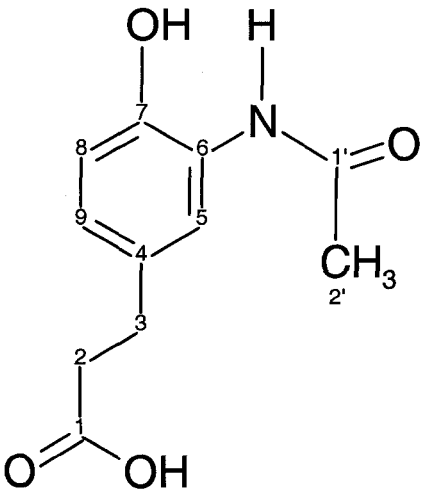


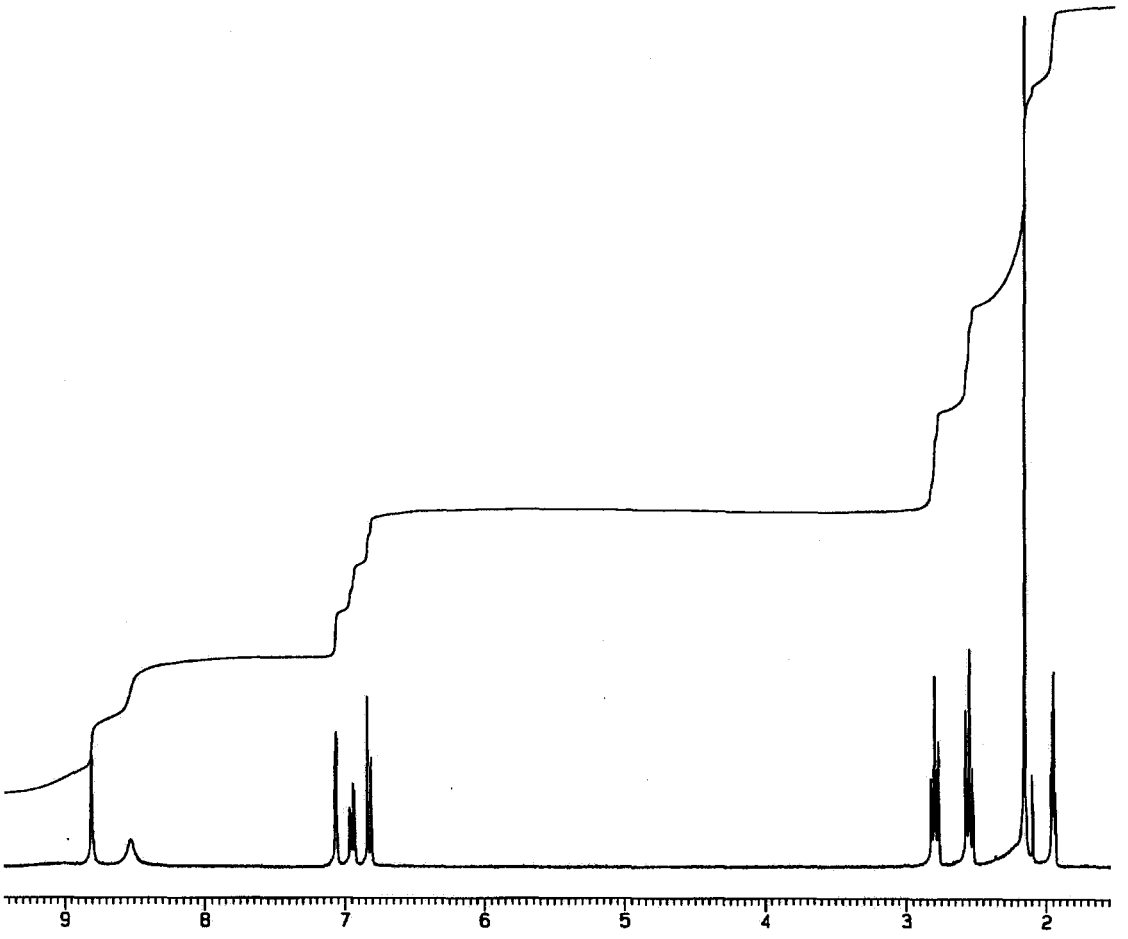
^{1}H-NMR Spectrum of Crude Compound **70**



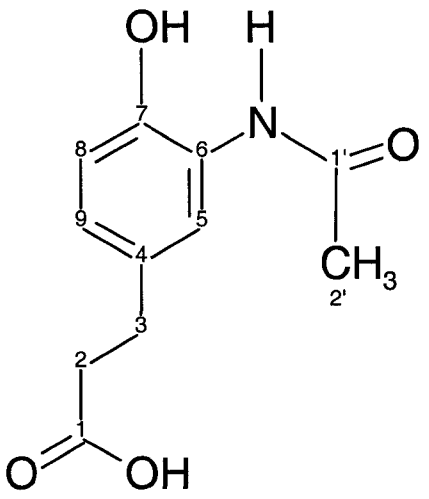


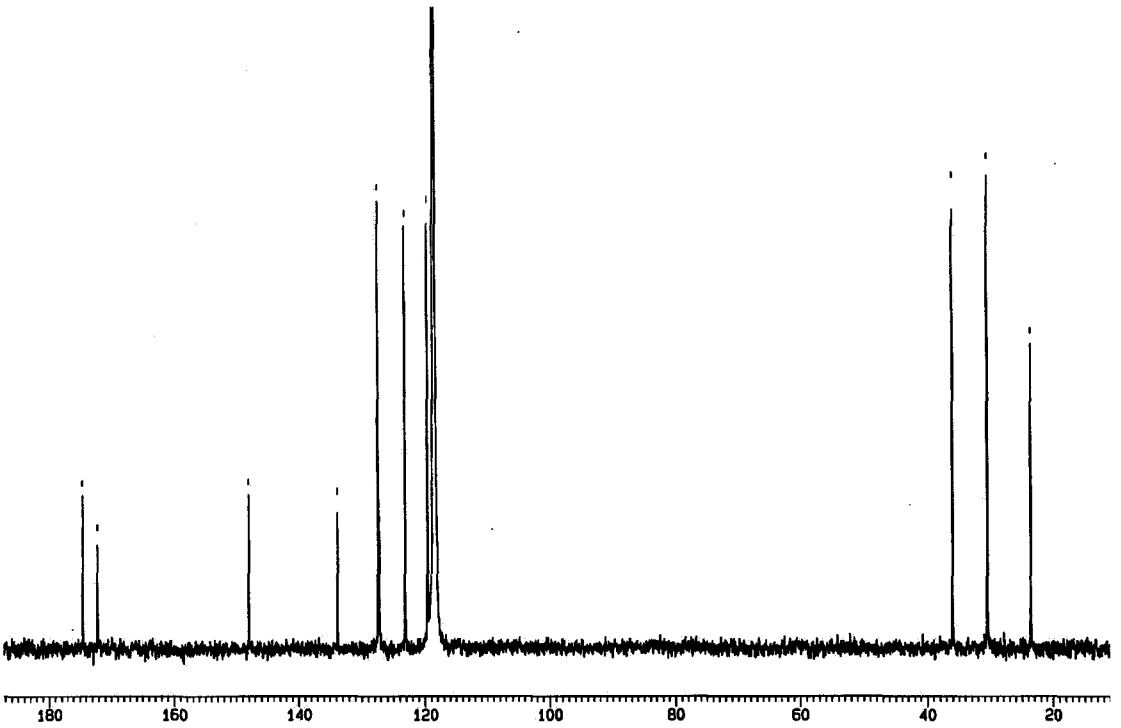
^{1}H-NMR Spectrum of Compound **71**



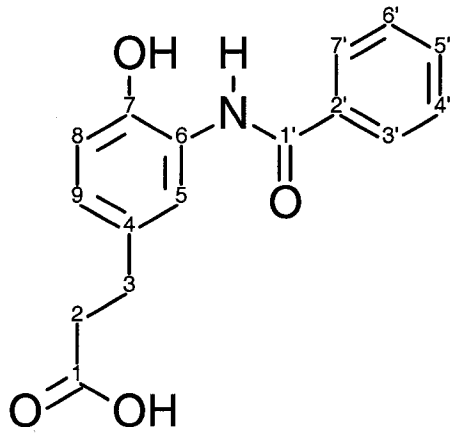


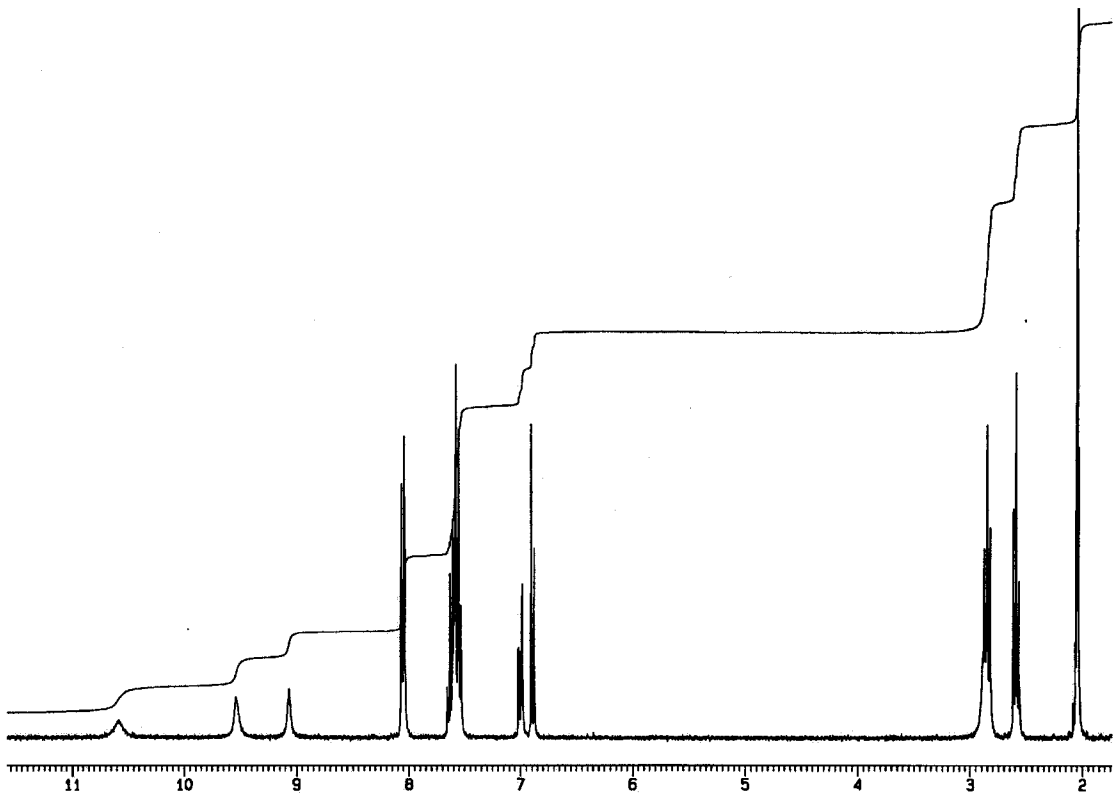
^{13}C-NMR Spectrum of Compound **71**



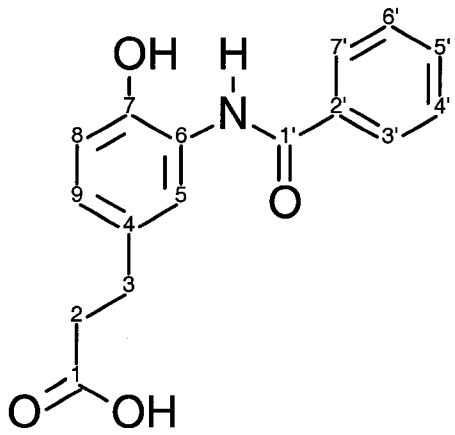


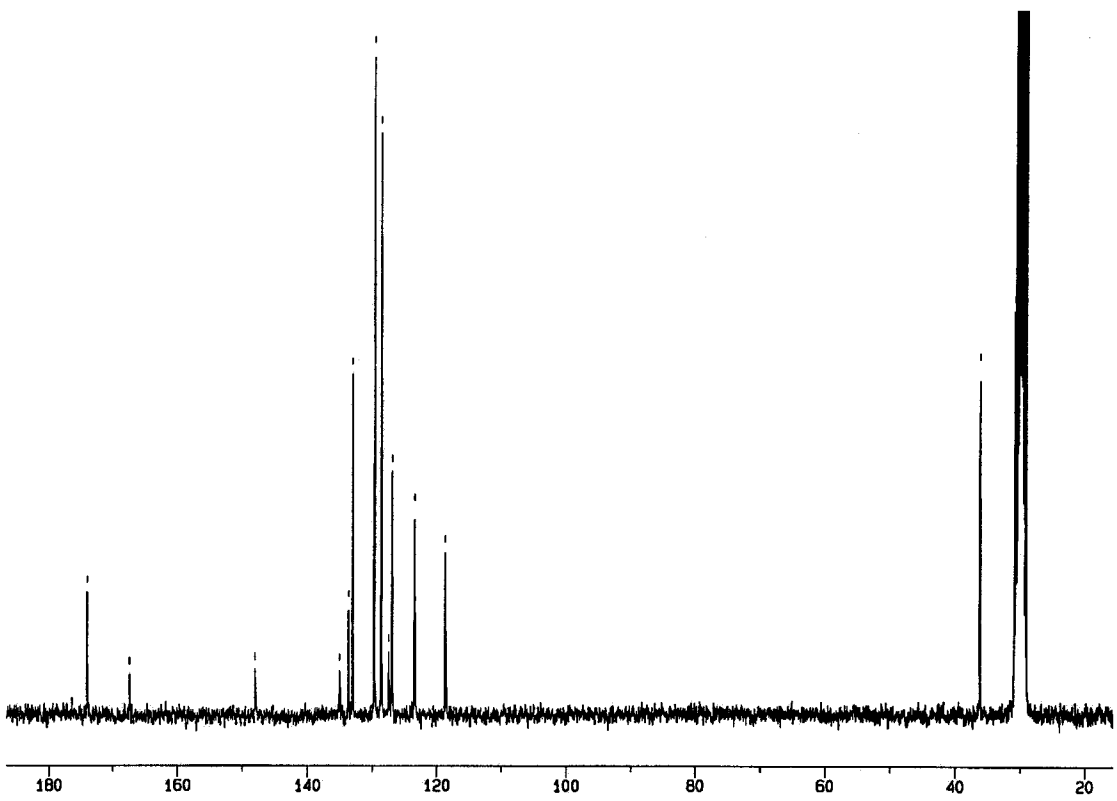
^{1}H-NMR Spectrum of Compound **72**



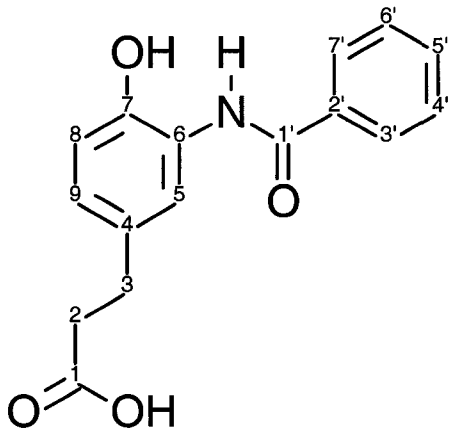


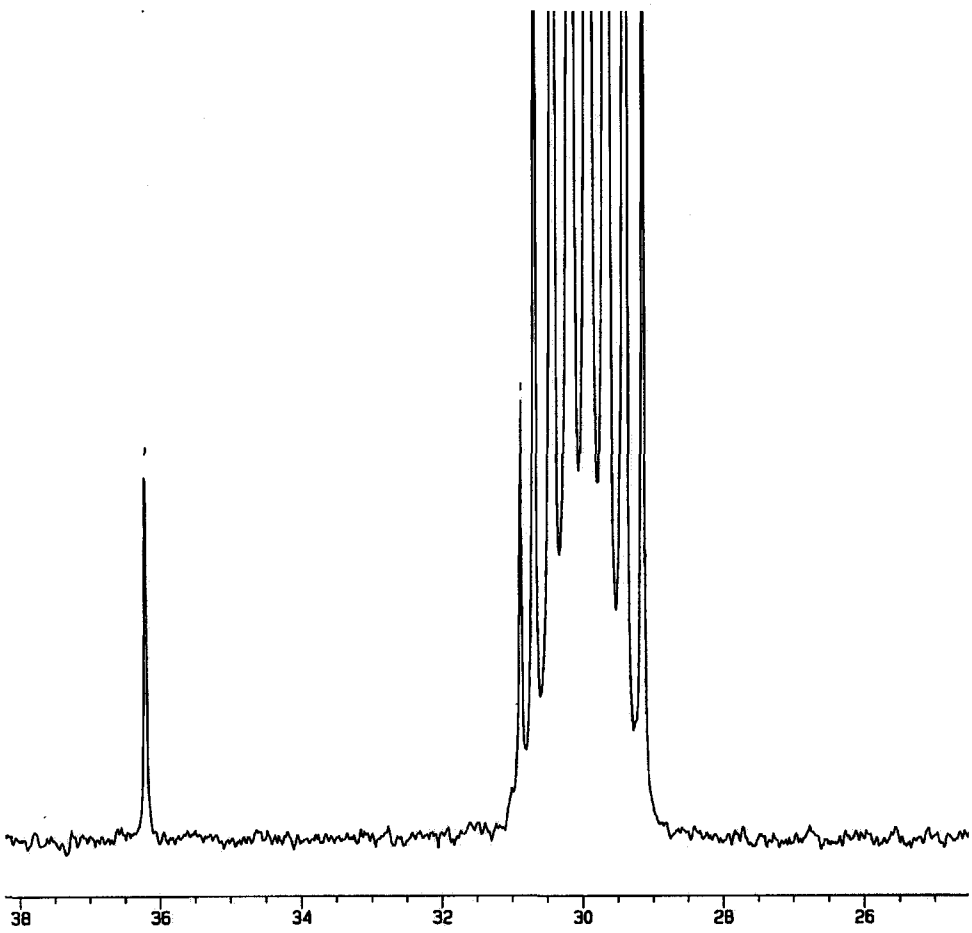
^{13}C-NMR Spectrum of Compound **72**



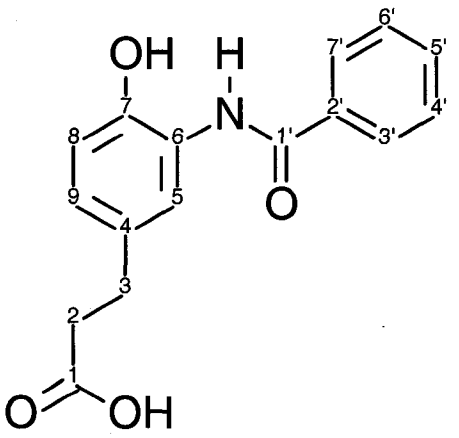


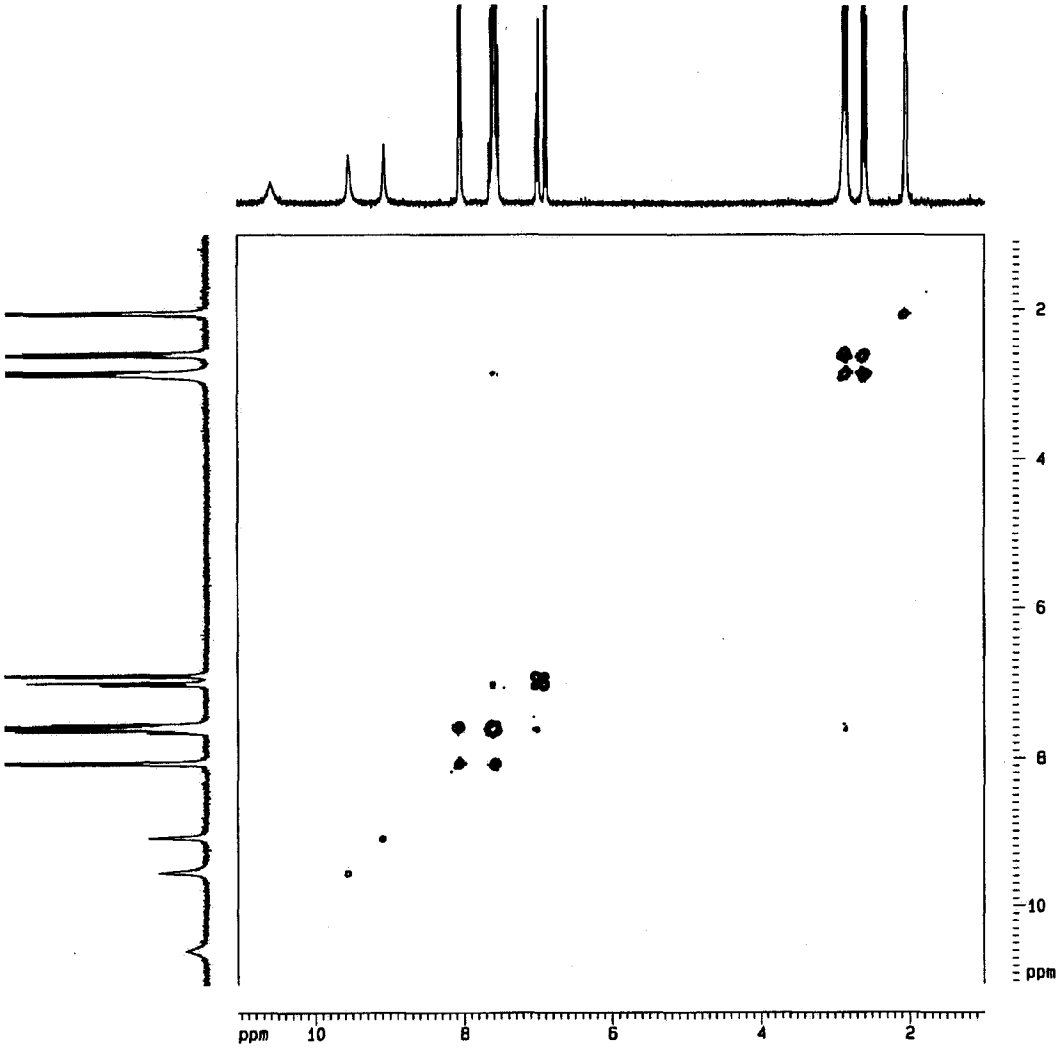
^{13}C-NMR Spectrum of Compound **72**



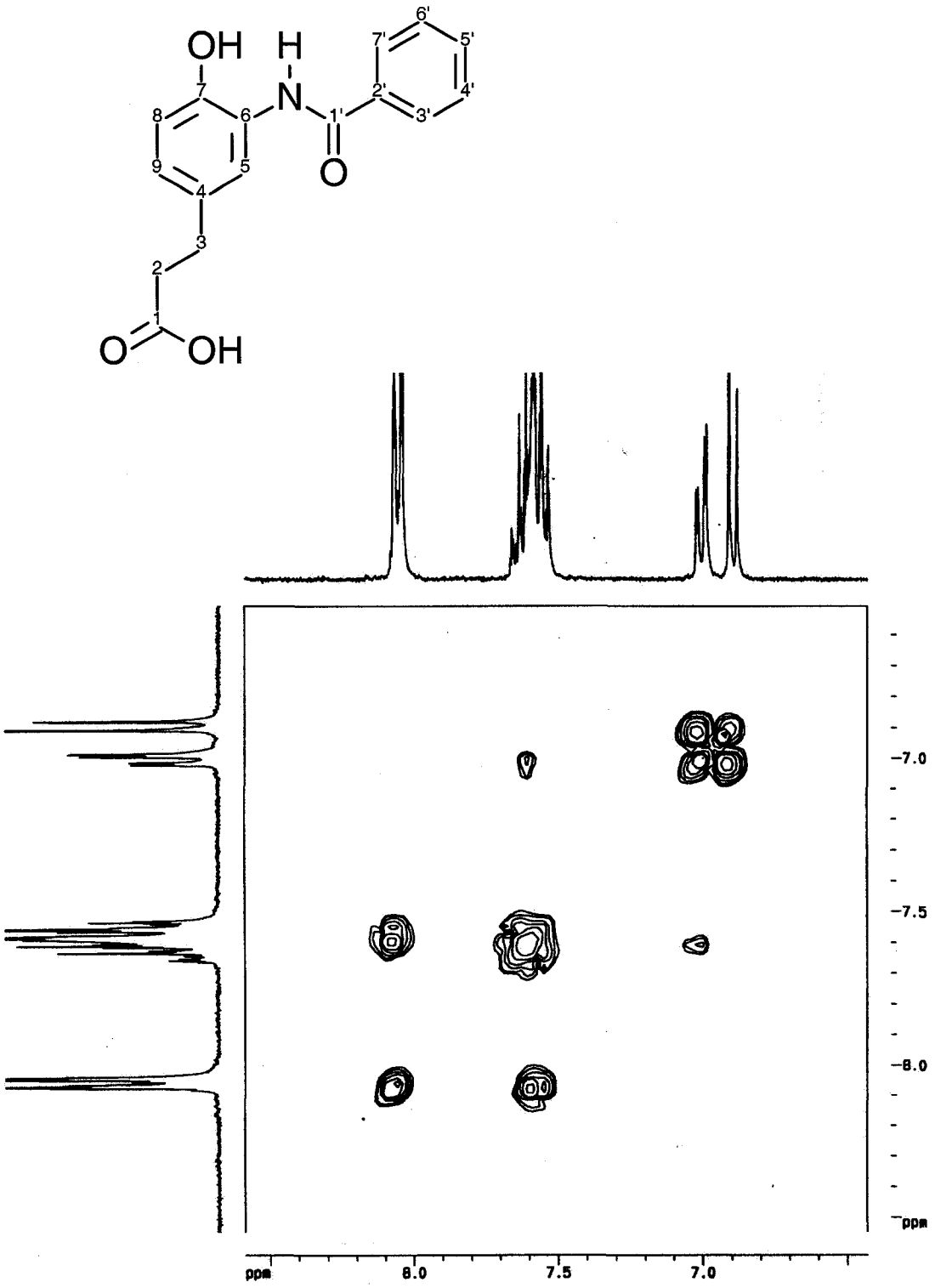


COSY-NMR Spectrum of Compound **72**

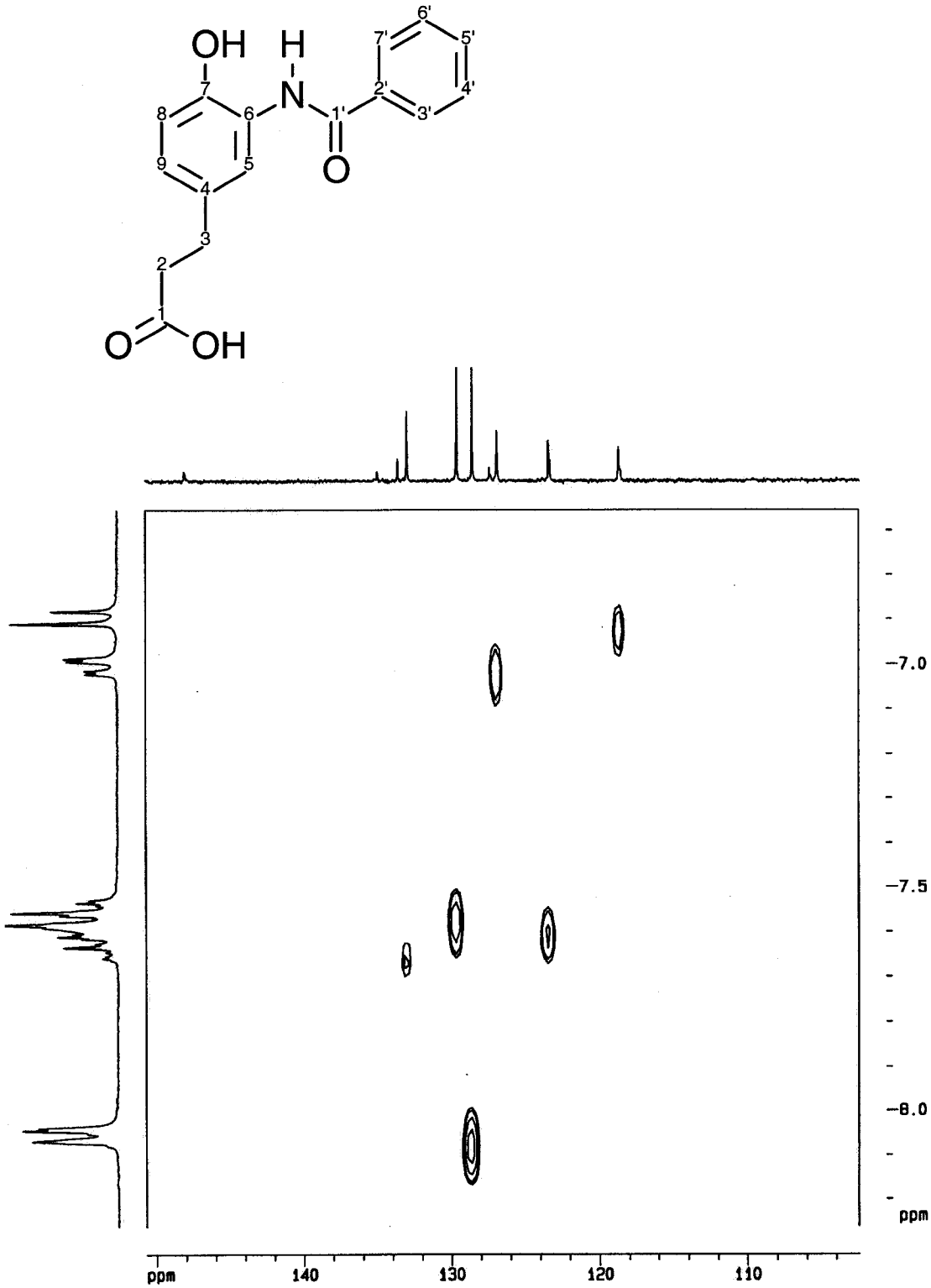




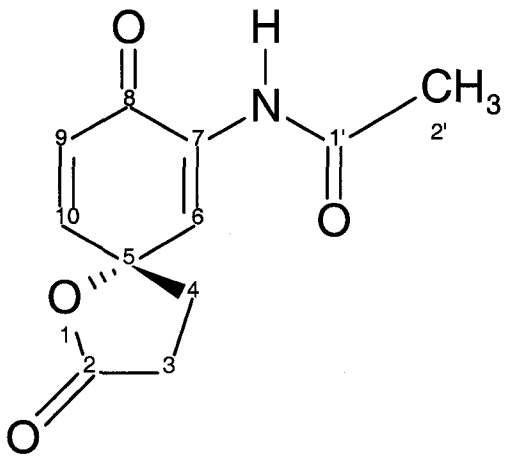
COSY-NMR Spectrum of Compound **72**

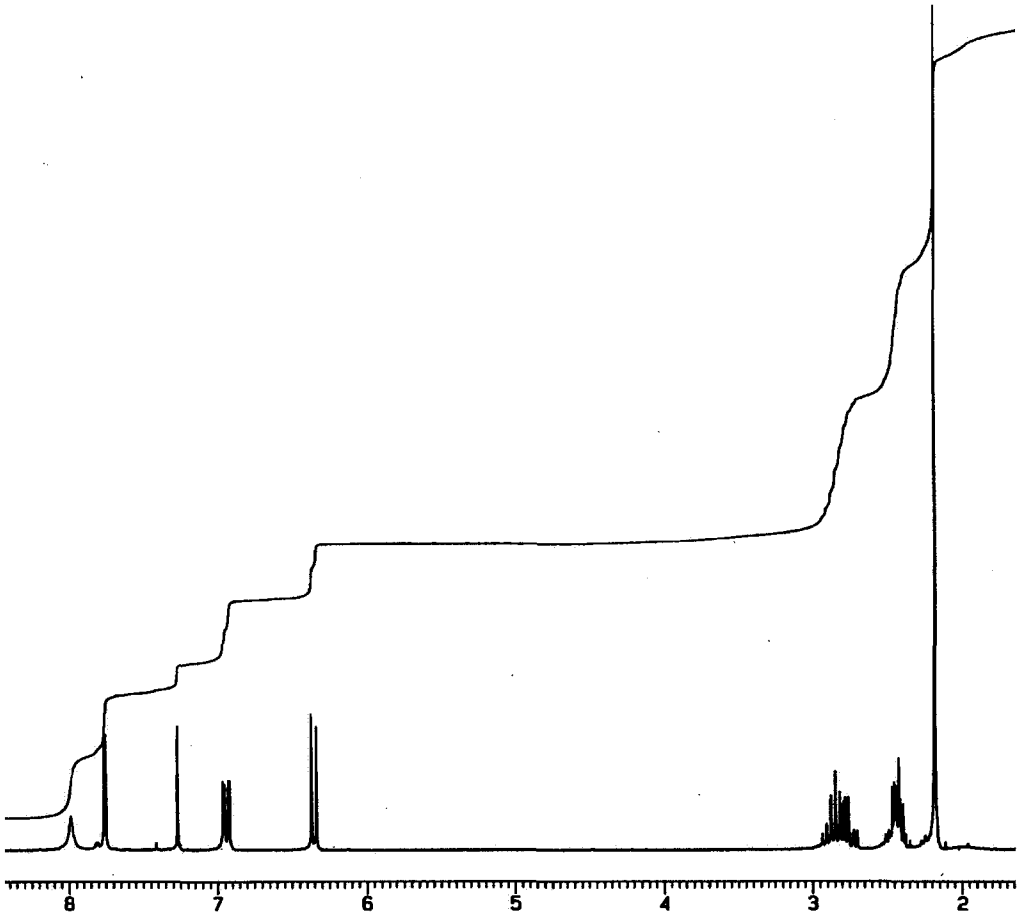


HETCOR-NMR Spectrum of Compound **72**

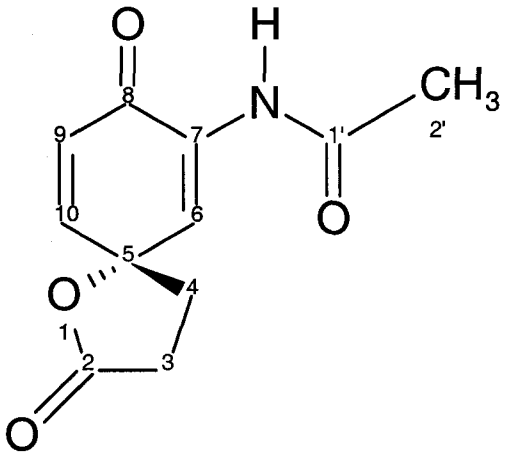


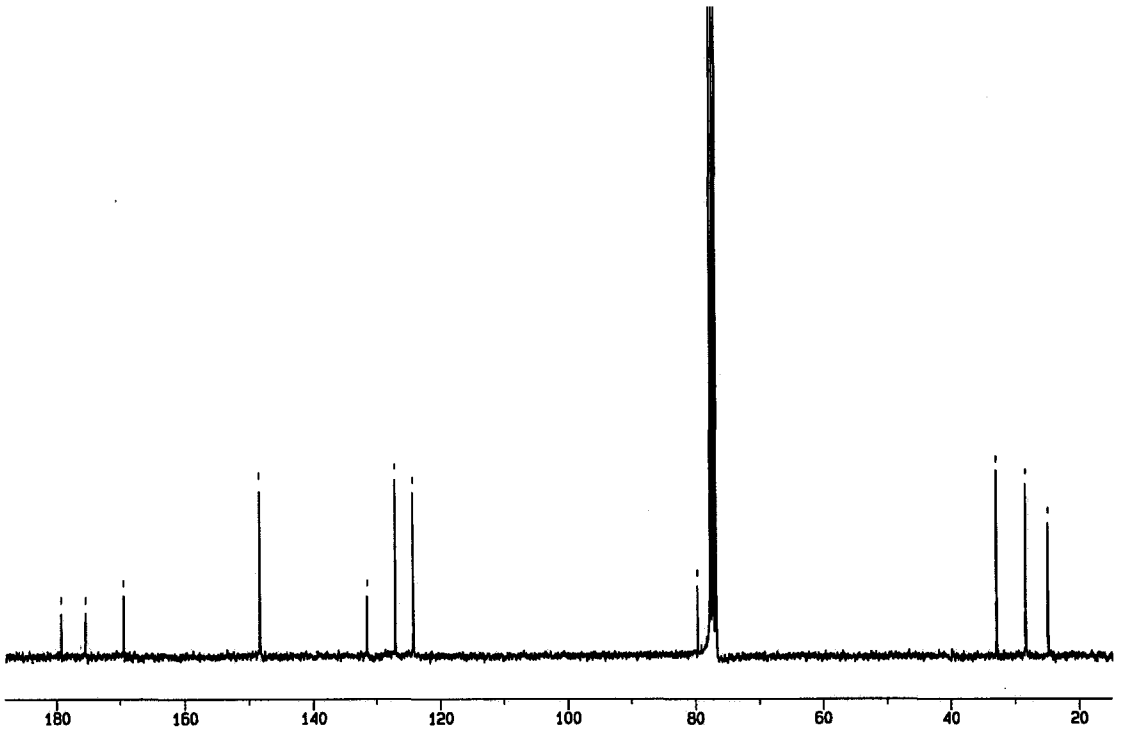
^{1}H-NMR Spectrum of Compound (+/-)-**73**



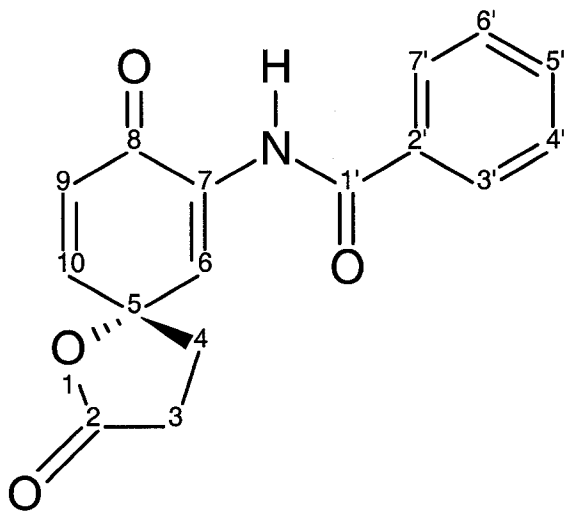


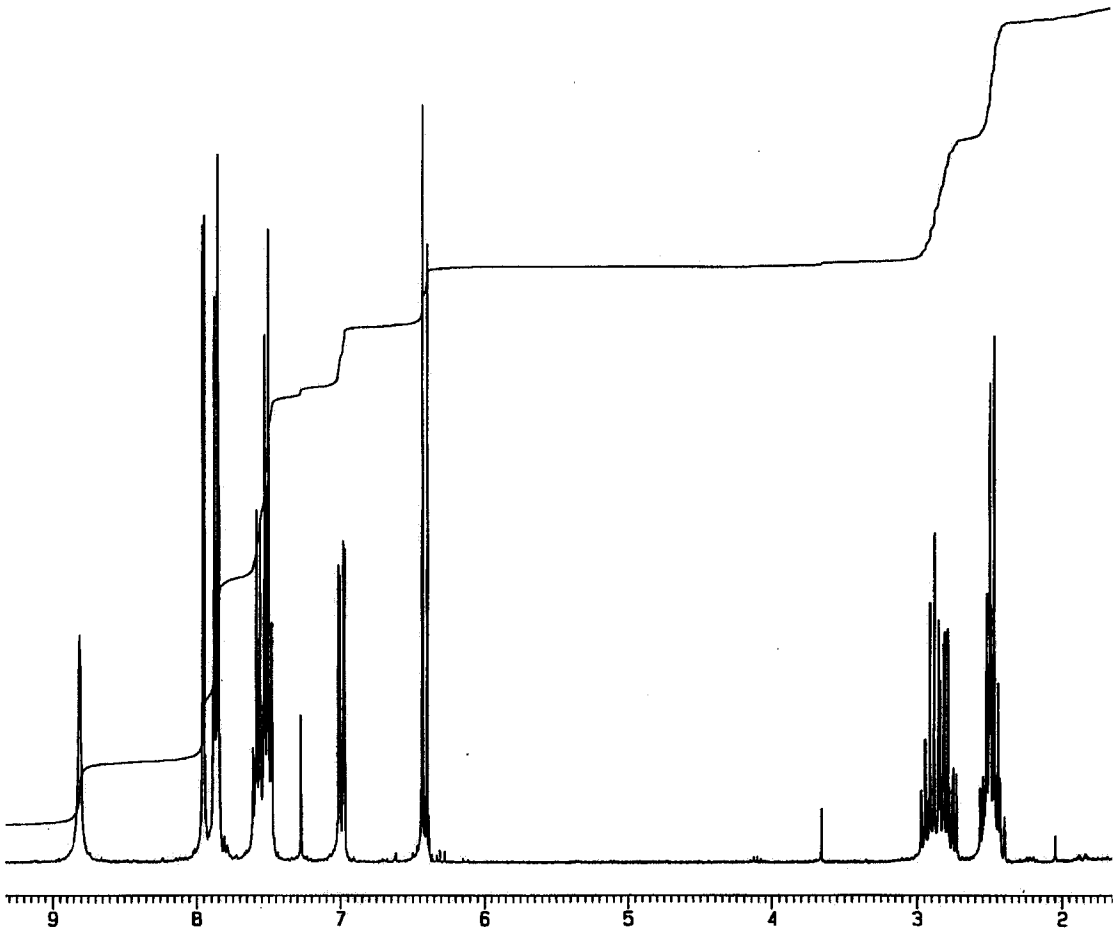
^{13}C-NMR Spectrum of Compound (+/-)-**73**



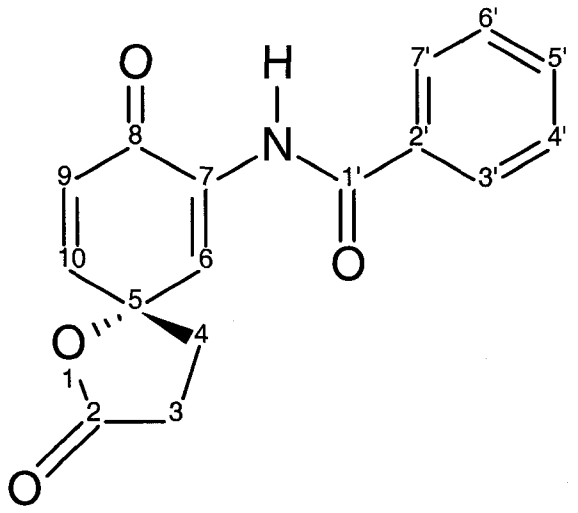


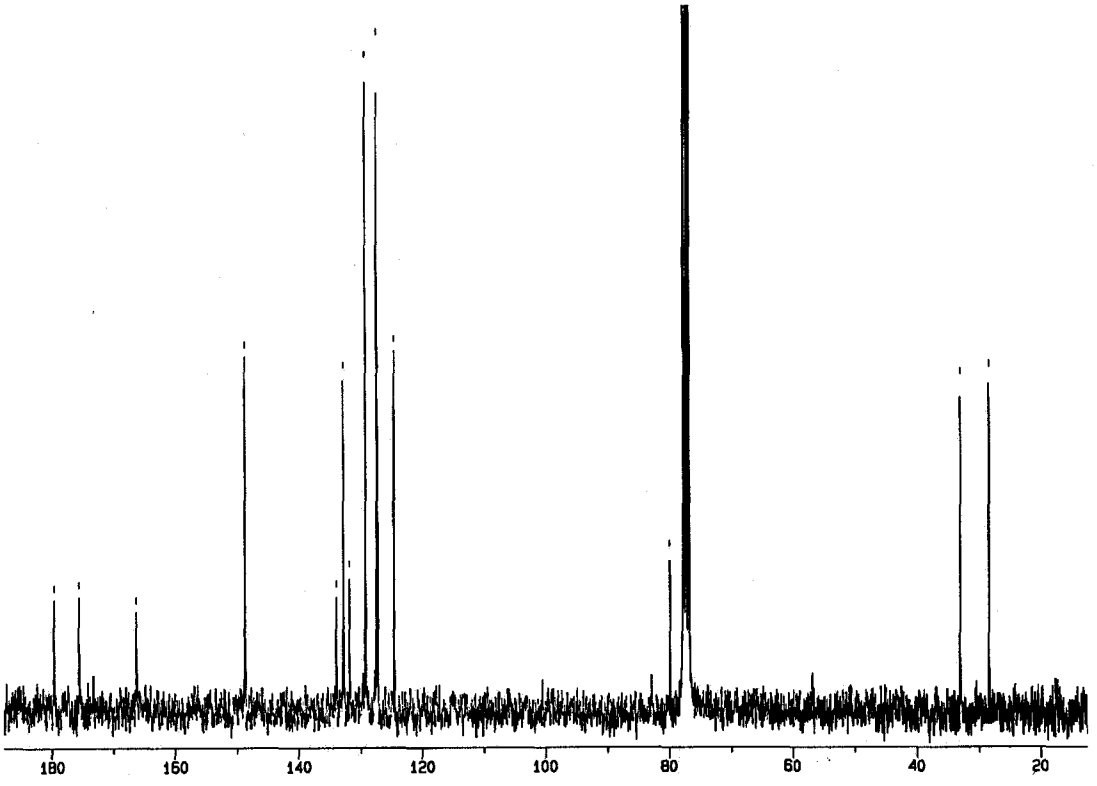
[1]H-NMR Spectrum of Compound (+/-)-**74**



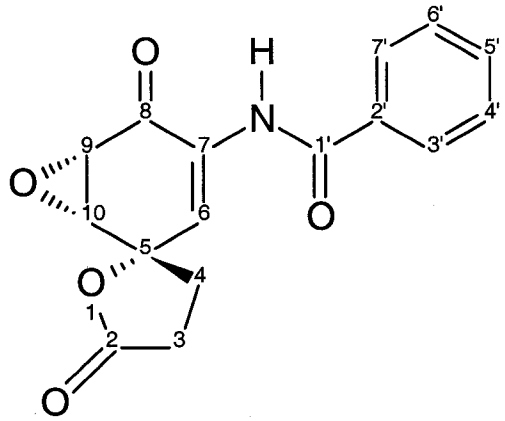


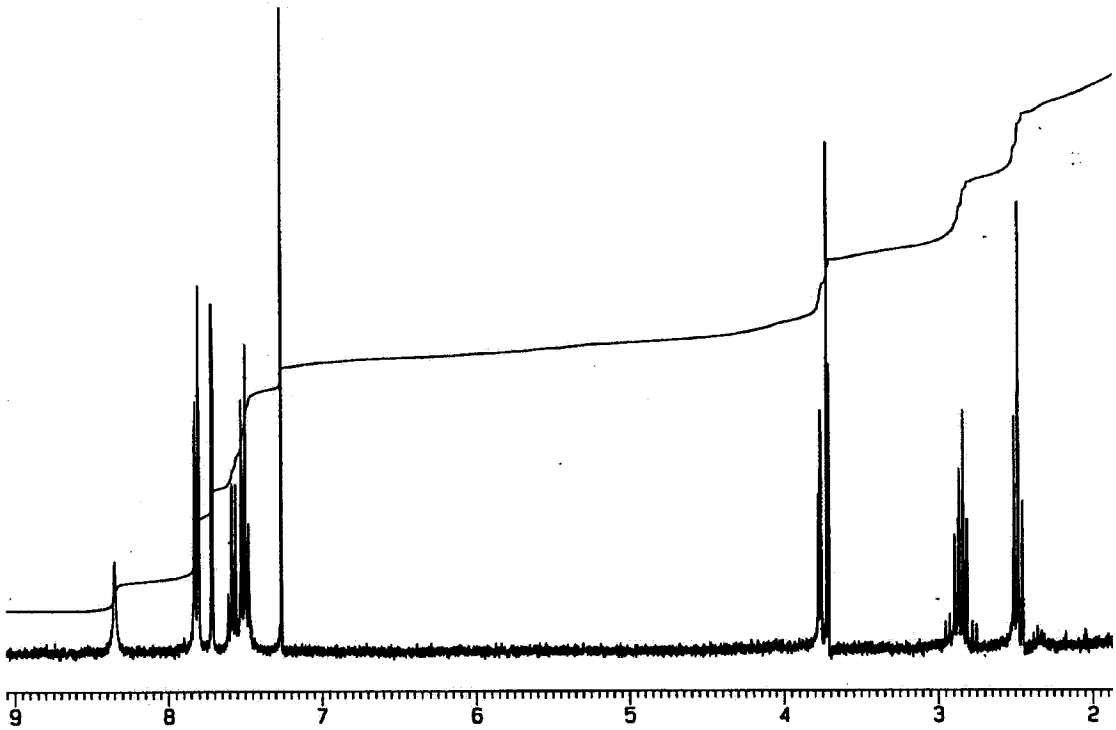
^{13}C-NMR Spectrum of Compound (+/-)-**74**



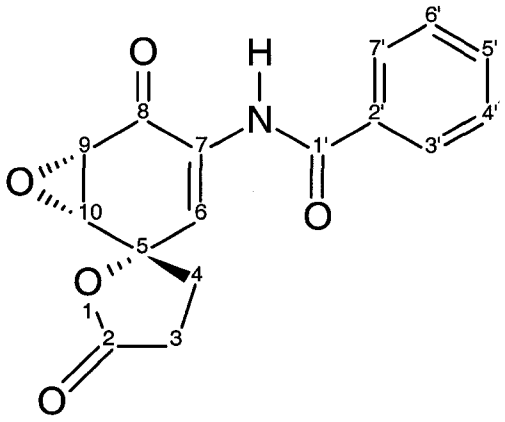


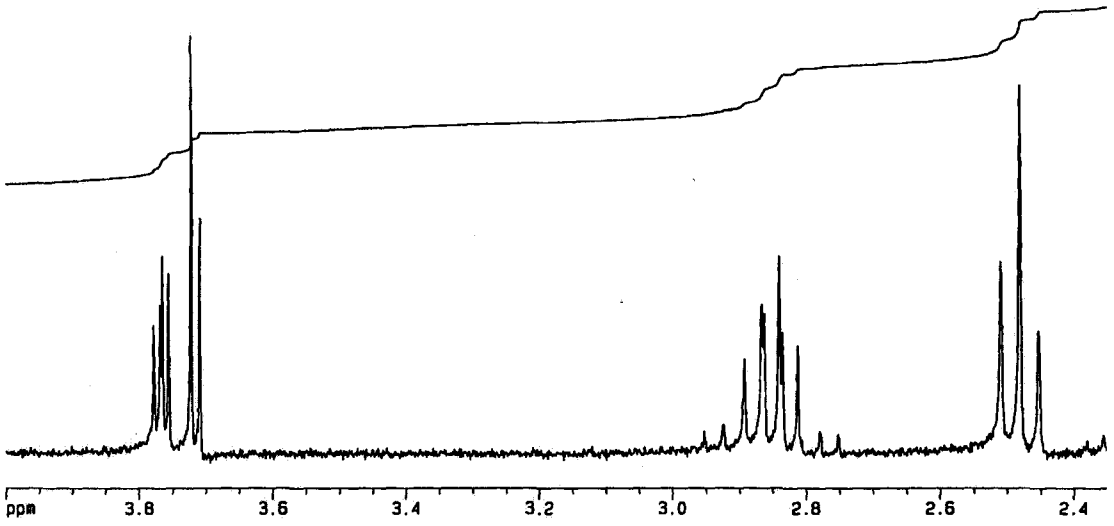
^{1}H-NMR Spectrum of Compound (+/-)-**76**



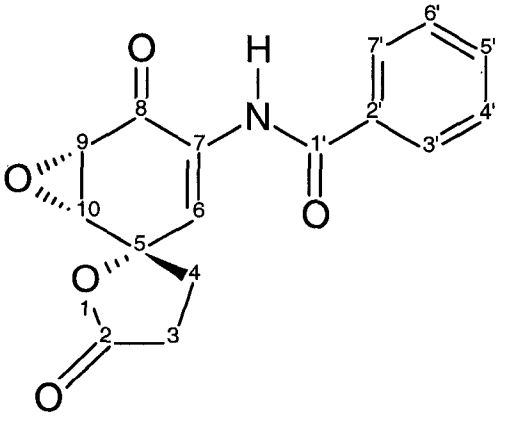


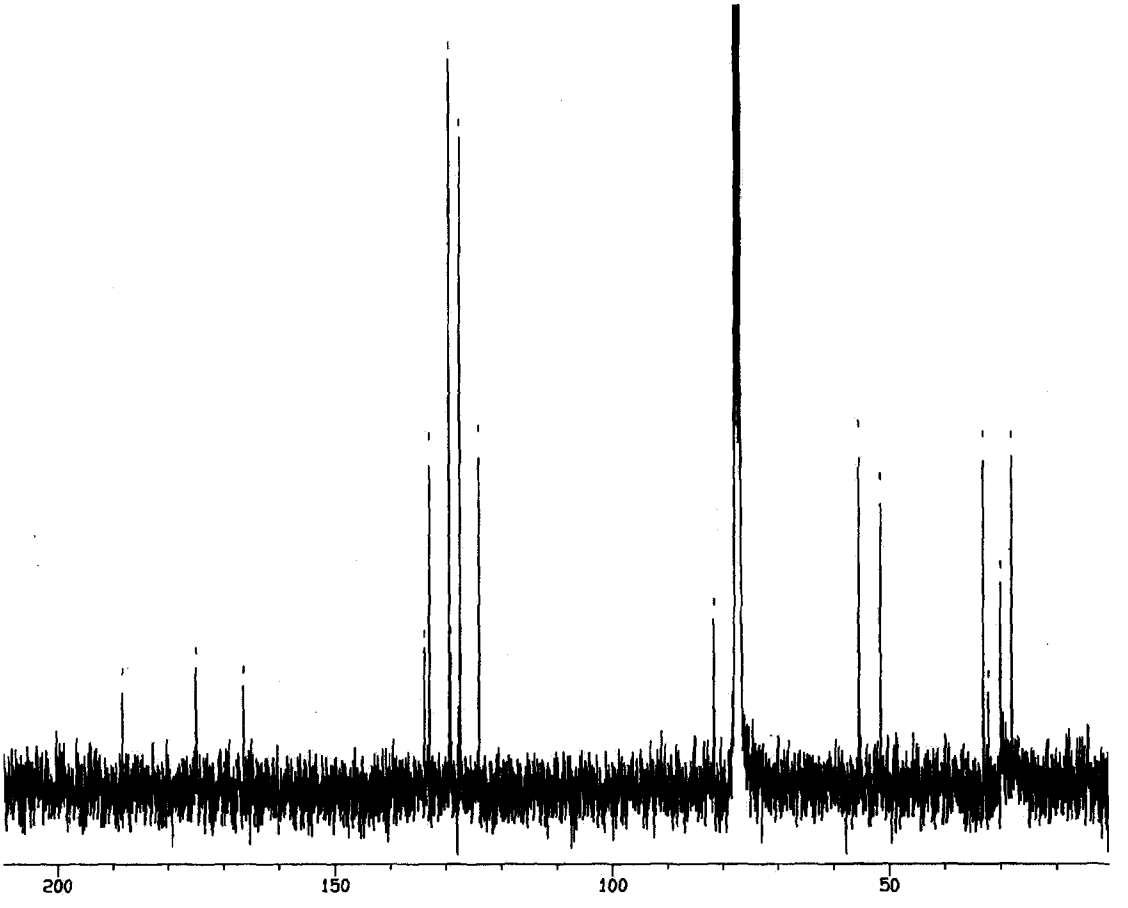
^{1}H-NMR Spectrum of Compound (+/-)-**76**



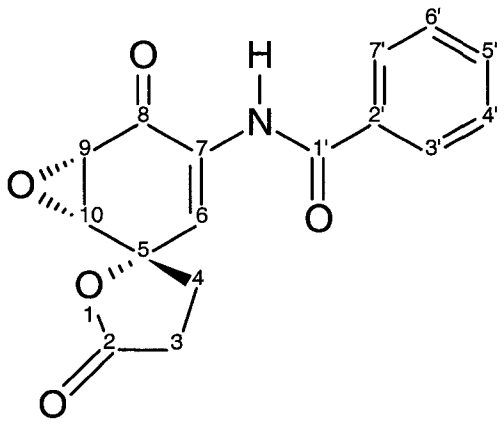


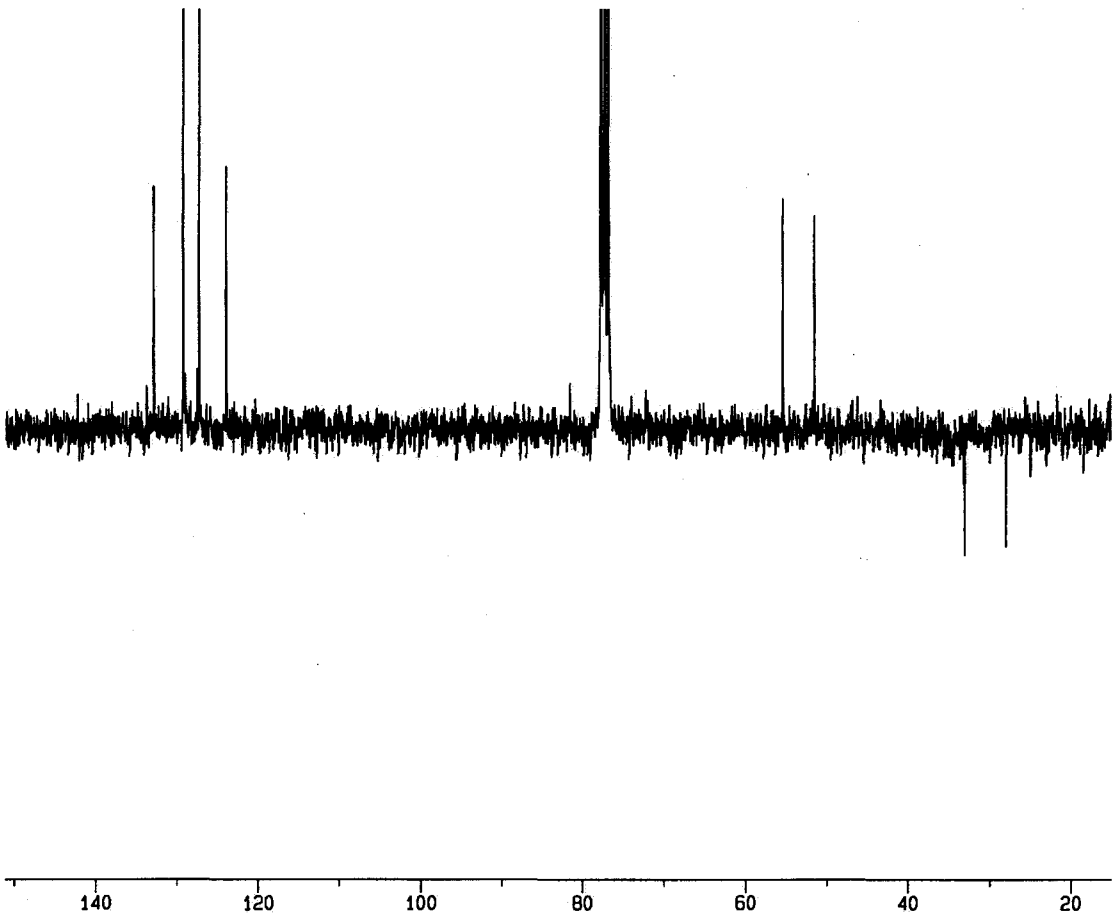
^{13}C-NMR Spectrum of Compound (+/-)-**76**



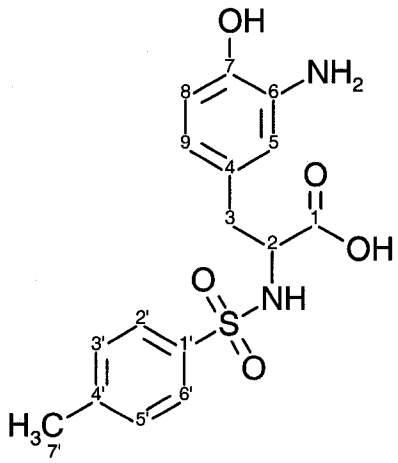


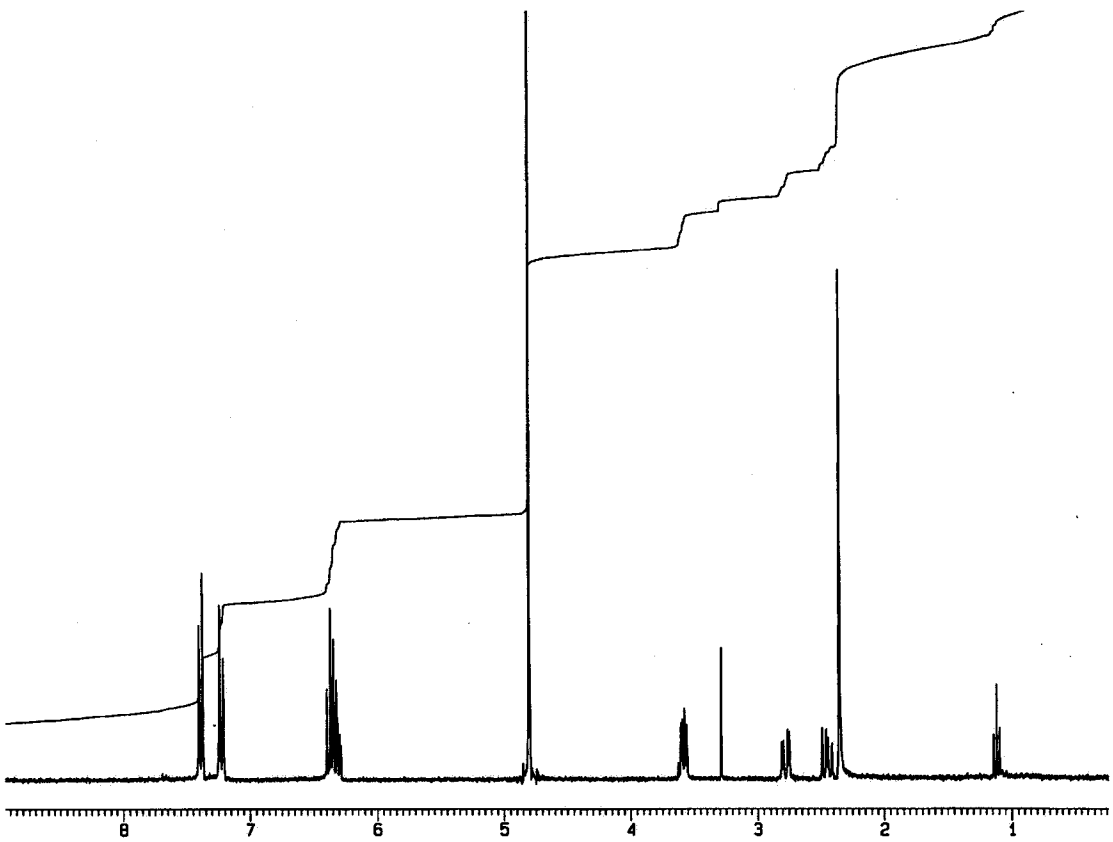
DEPT-135-NMR Spectrum of Compound (+/-)-**76**



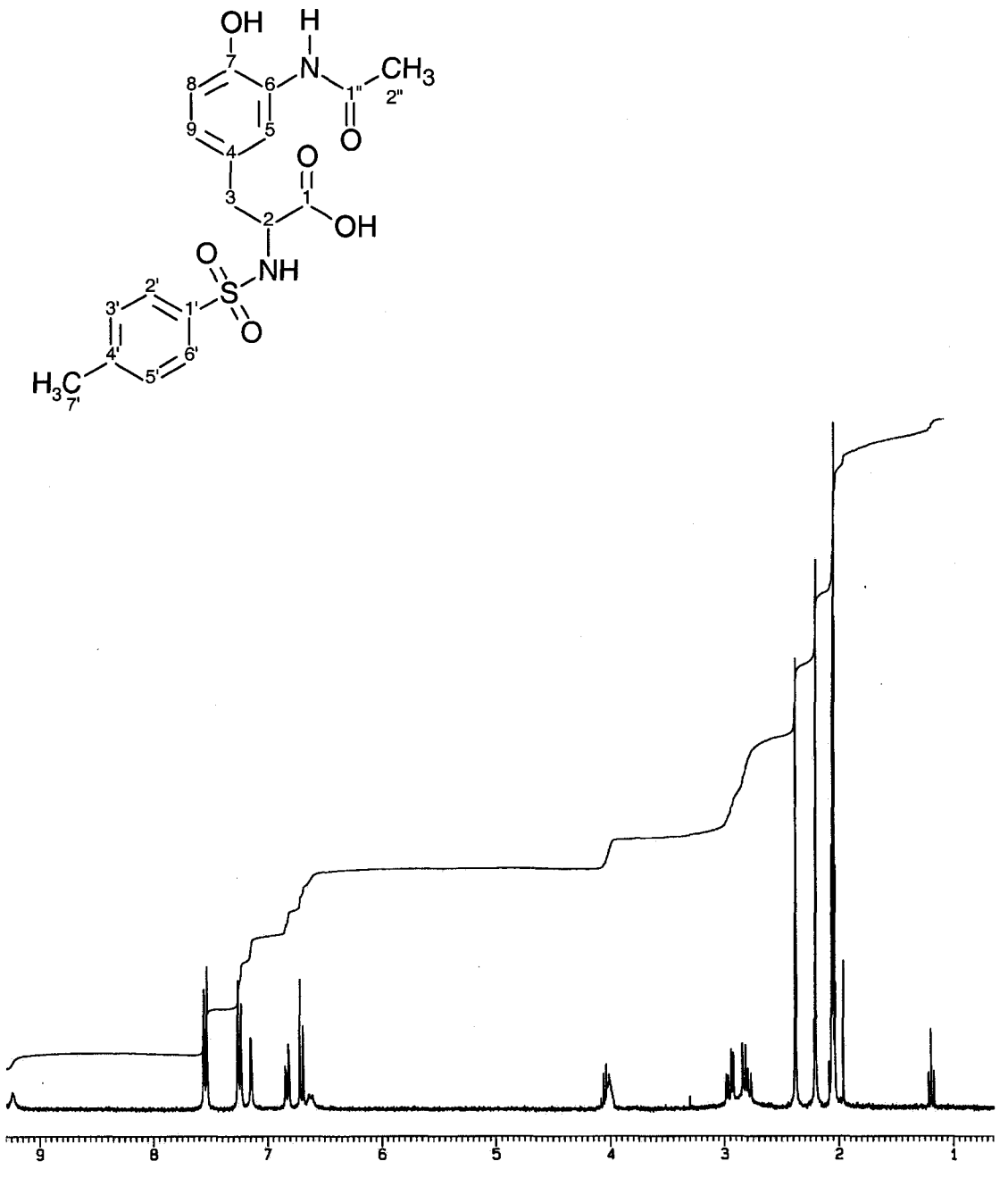


^{1}H-NMR Spectrum of Compound **77**

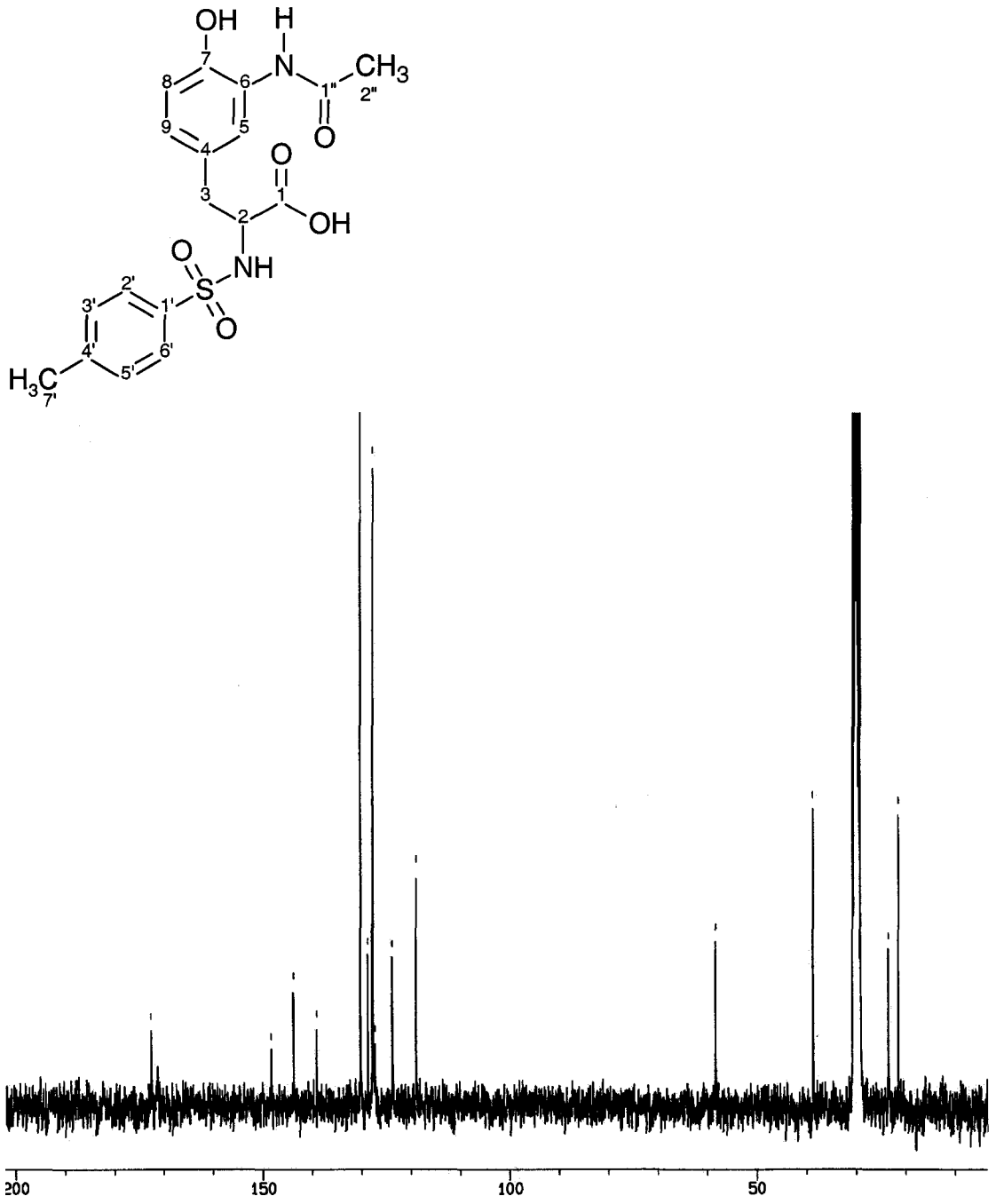




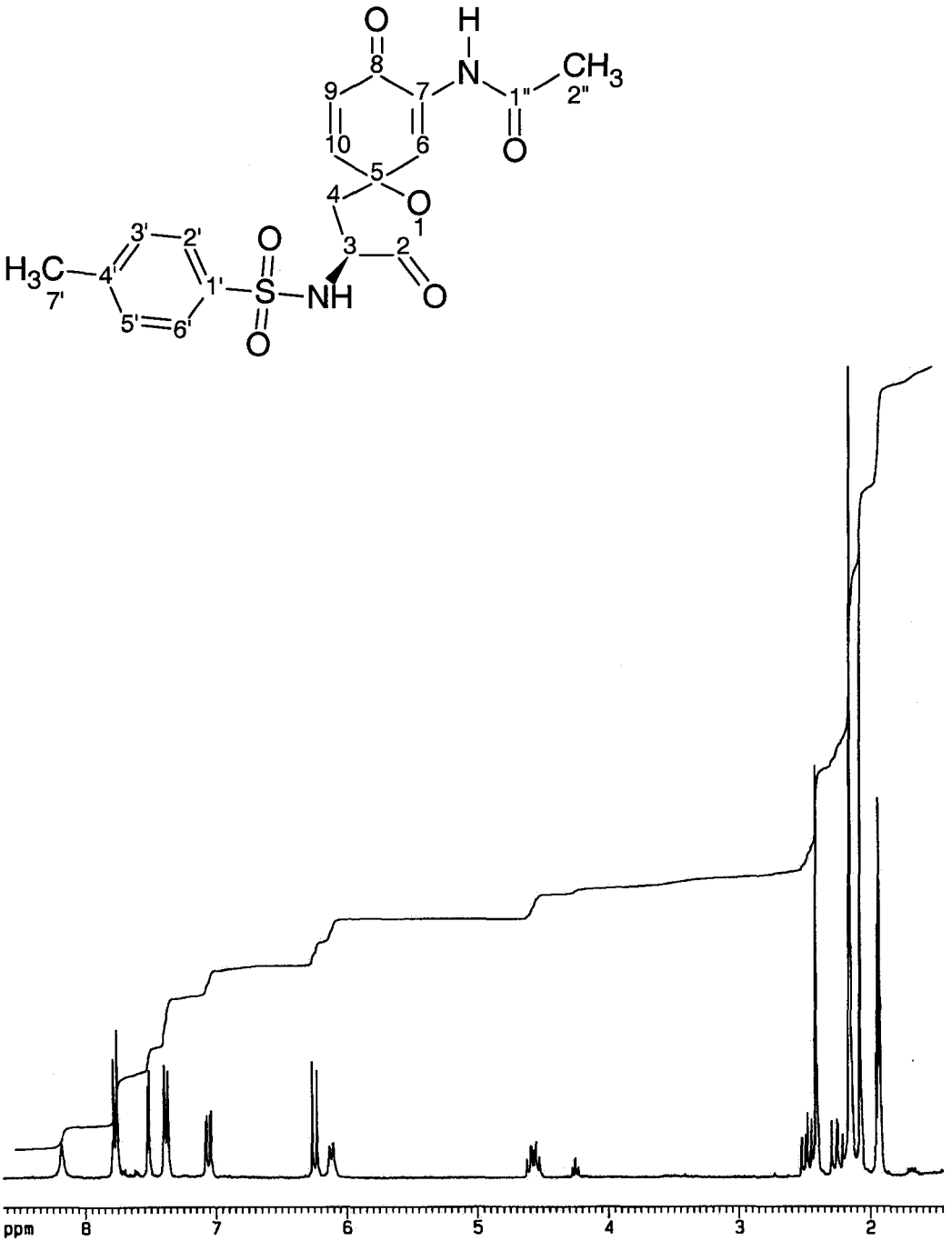
^{1}H-NMR Spectrum of Compound **78**



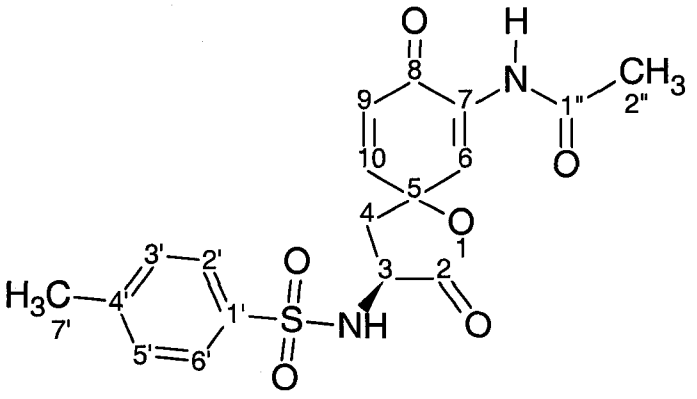
^{13}C-NMR Spectrum of Compound **78**

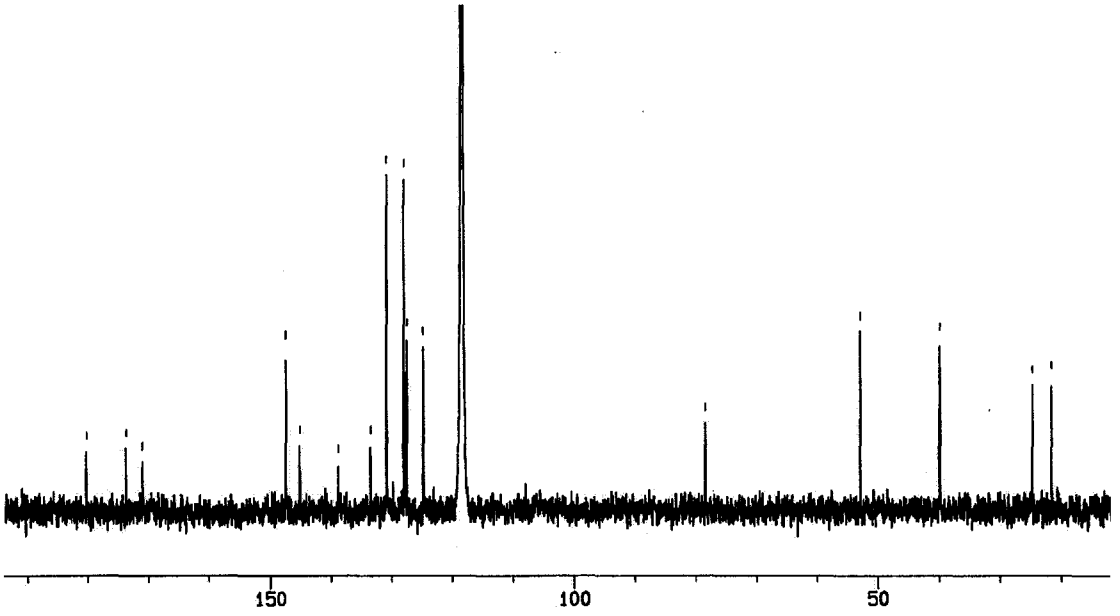


[1]H-NMR Spectrum of Compound (+)-**79**, Major Diastereomer

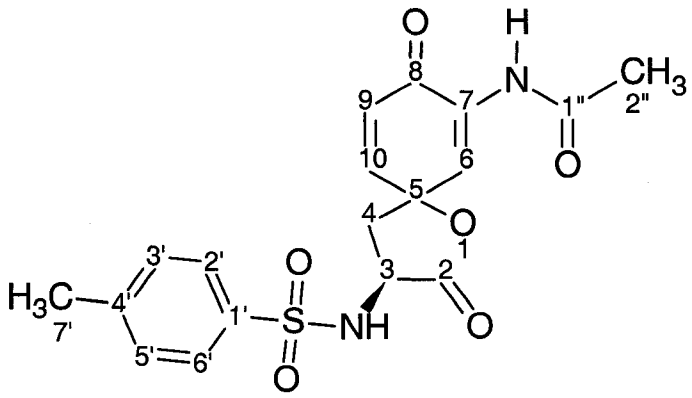


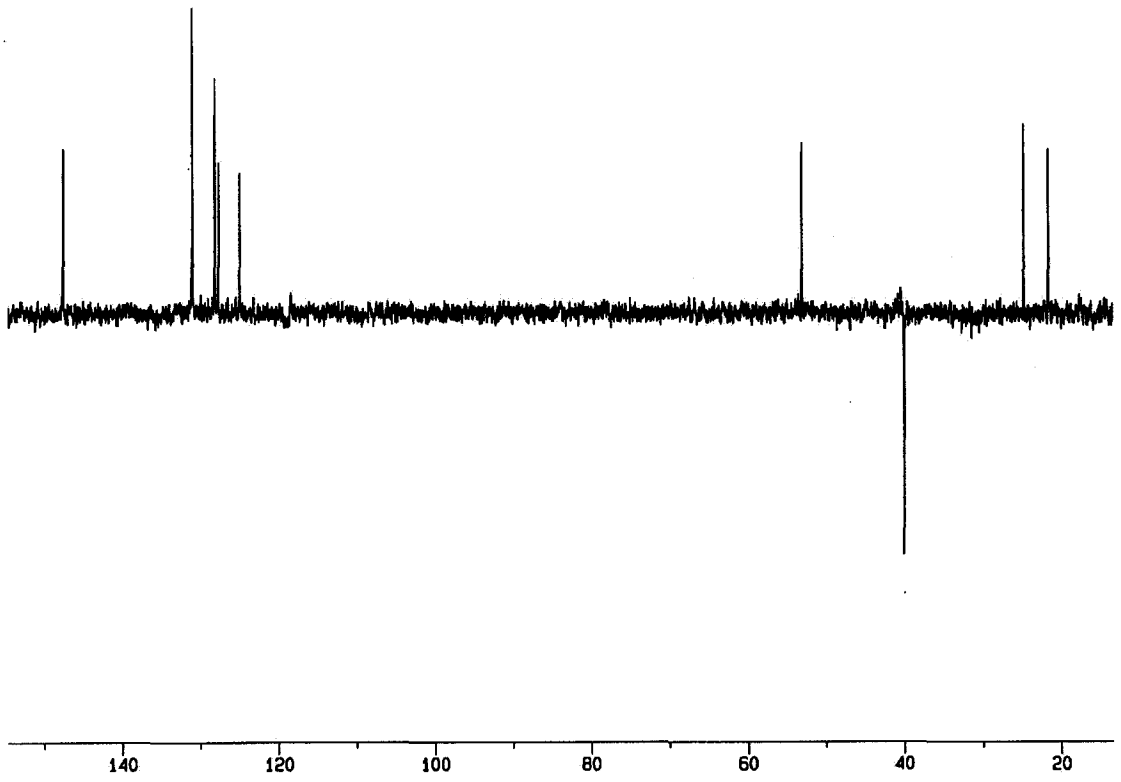
^{13}C-NMR Spectrum of Compound (+)-**79**, Major Diastereomer



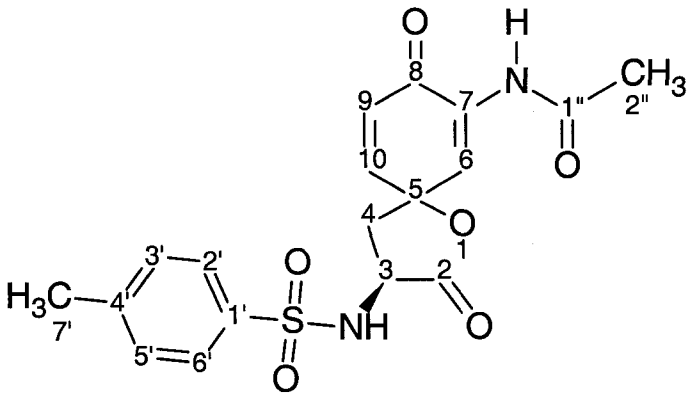


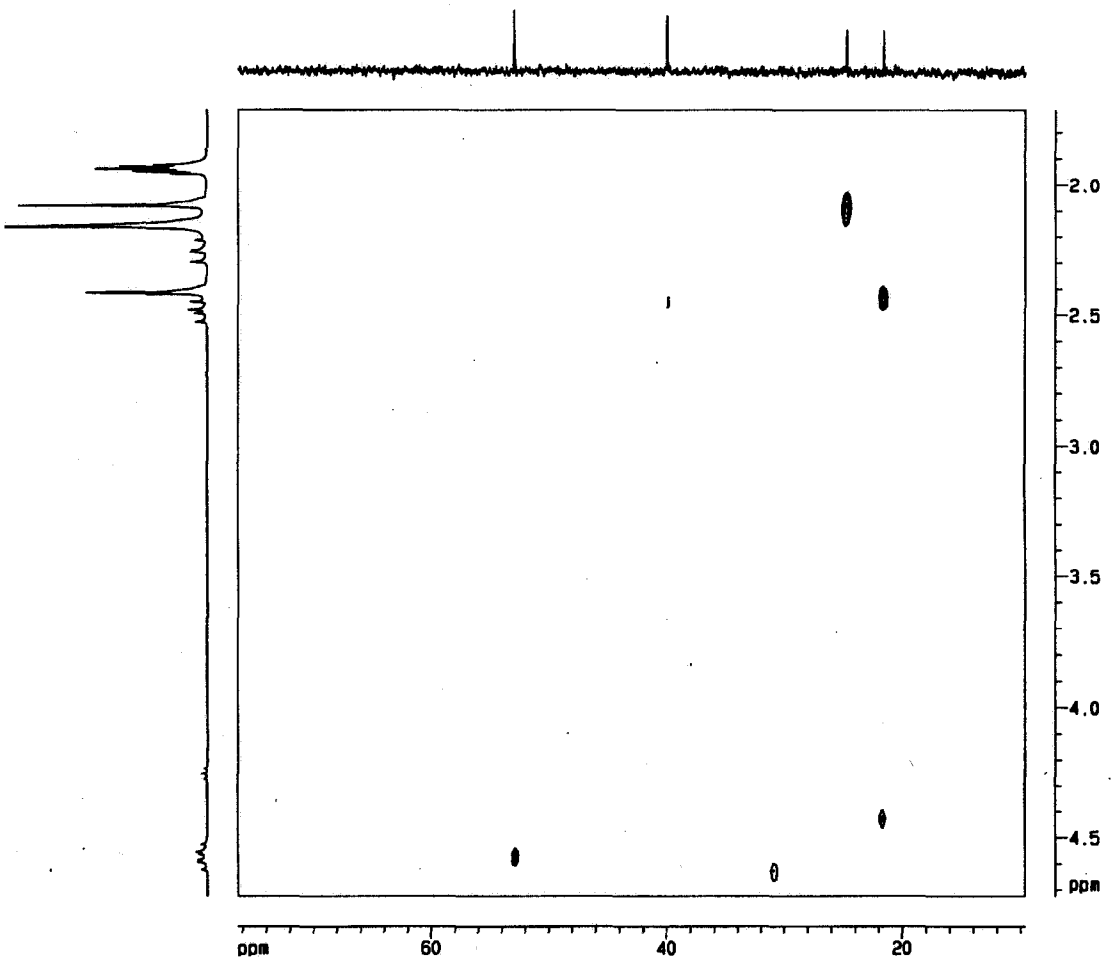
DEPT-NMR Spectrum of Compound (+)-**79**, Major Diastereomer



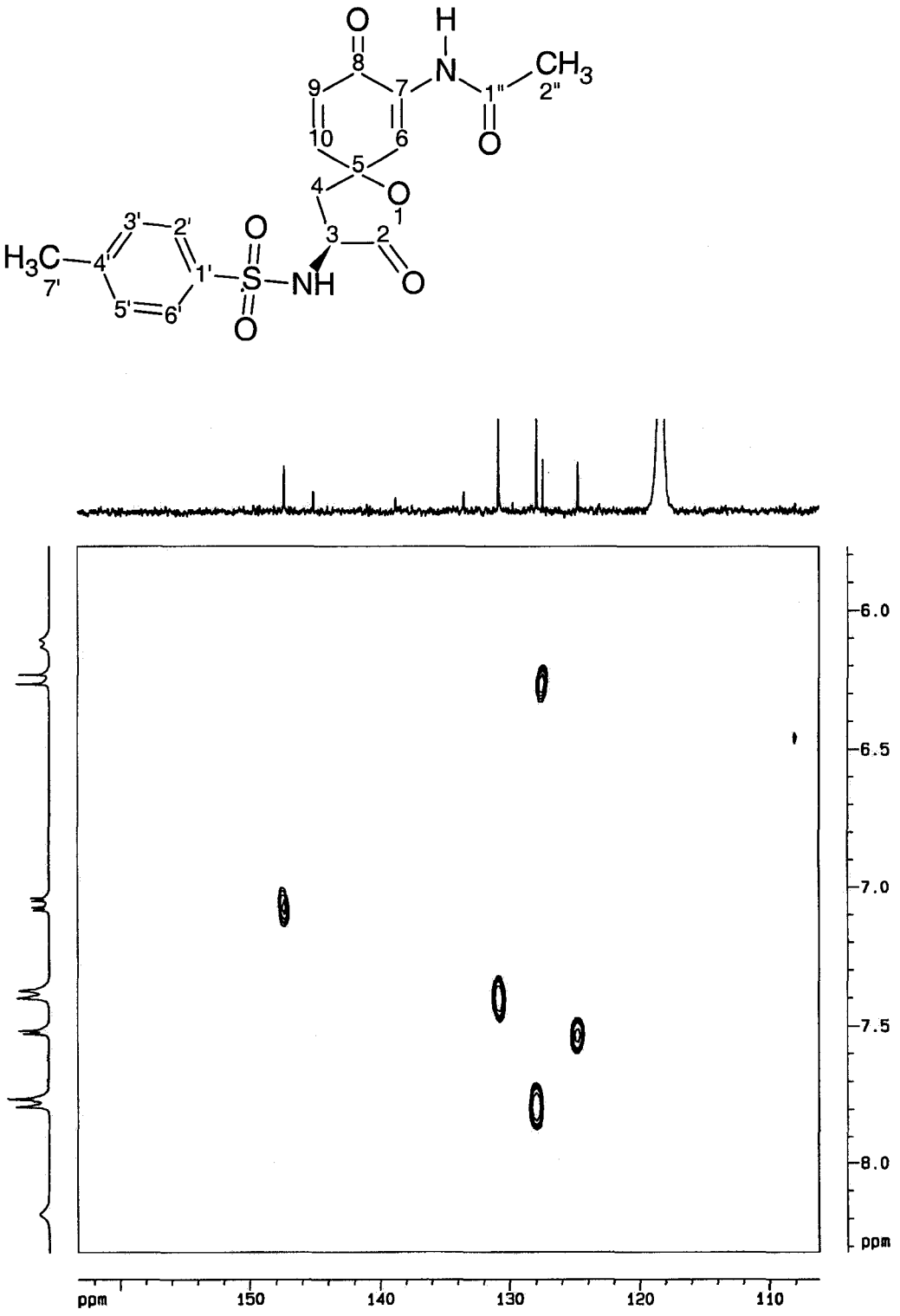


HETCOR-NMR Spectrum of Compound (+)-**79**, Major Diastereomer

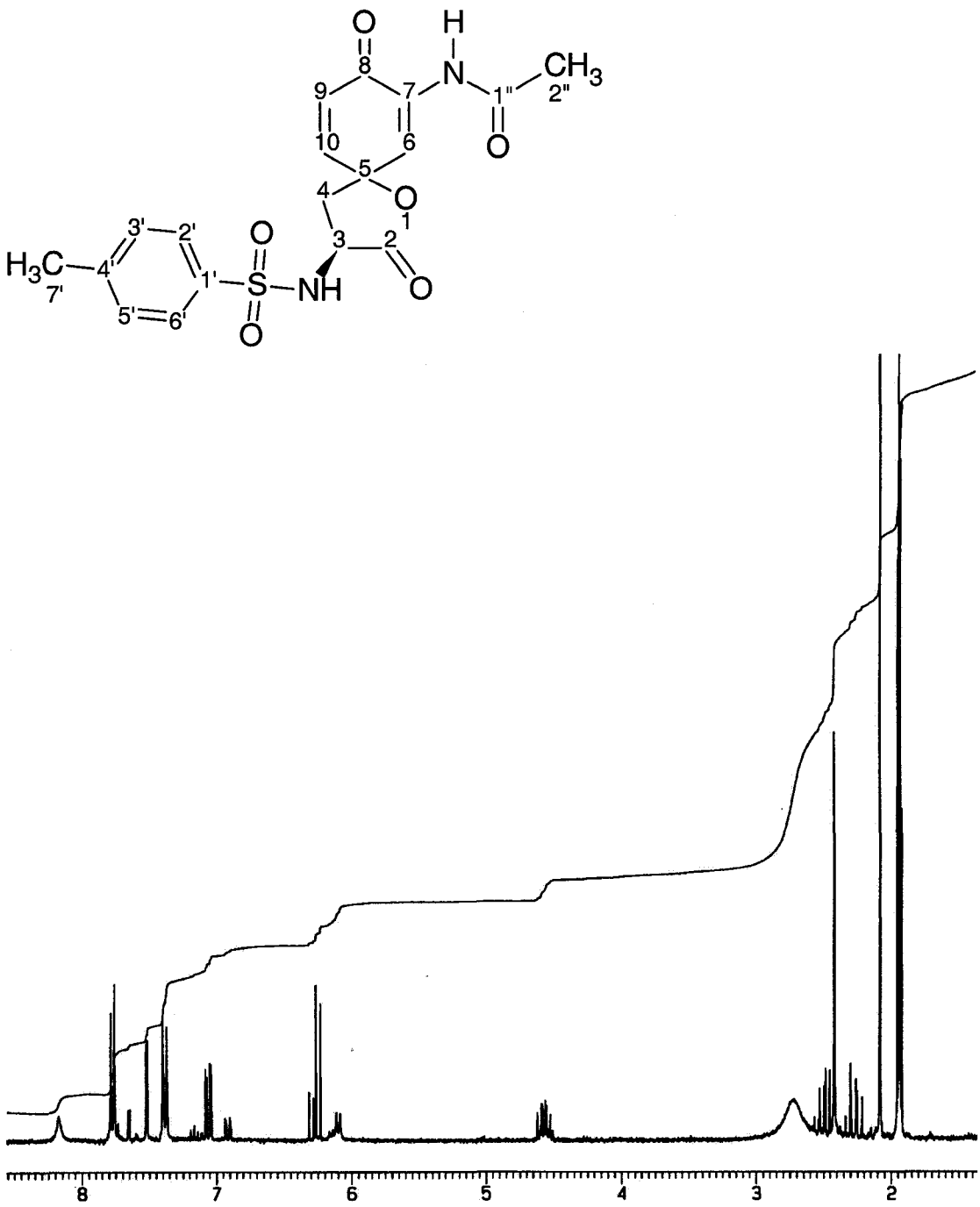




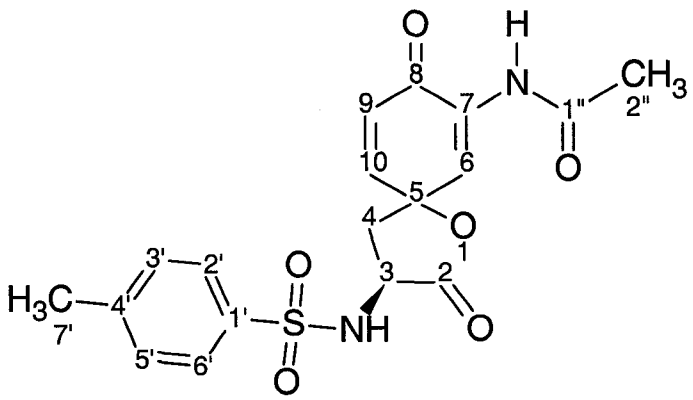
HETCOR-NMR Spectrum of Compound (+)-**79**, Major Diastereomer

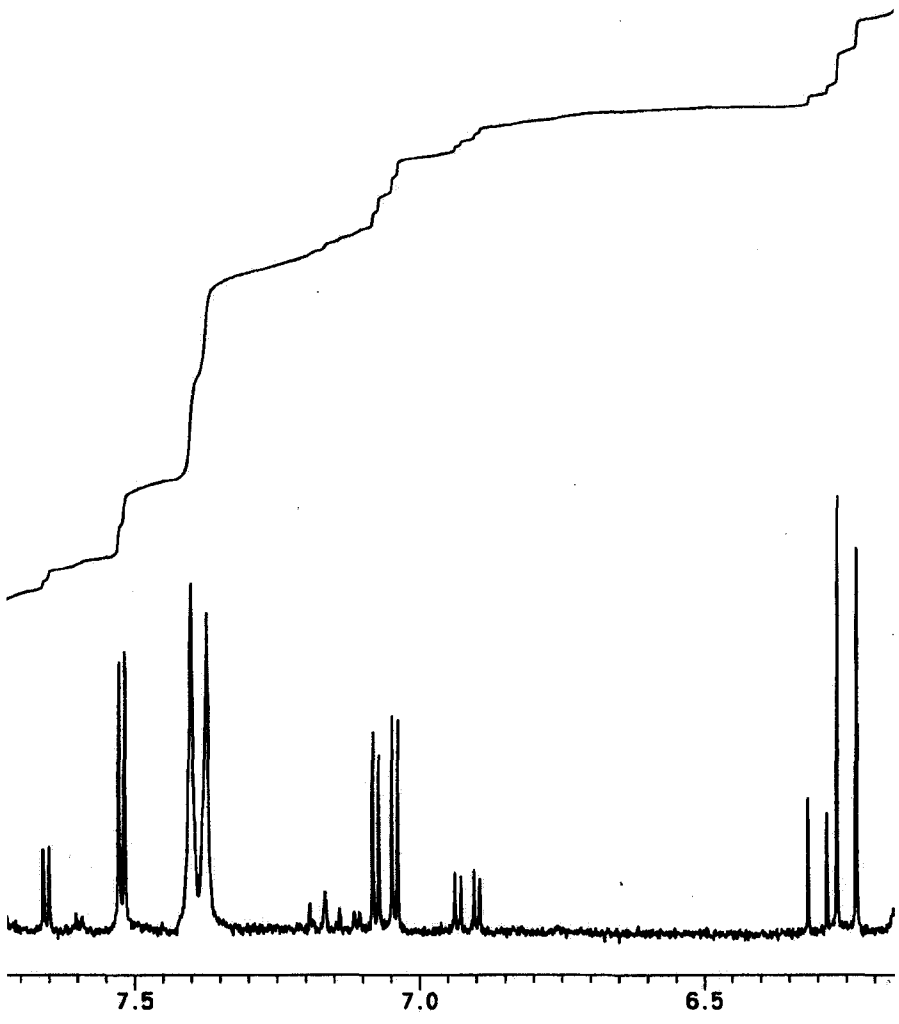


^{1}H-NMR Spectrum of Crude Product (+)-**79/80**, Diastereomers

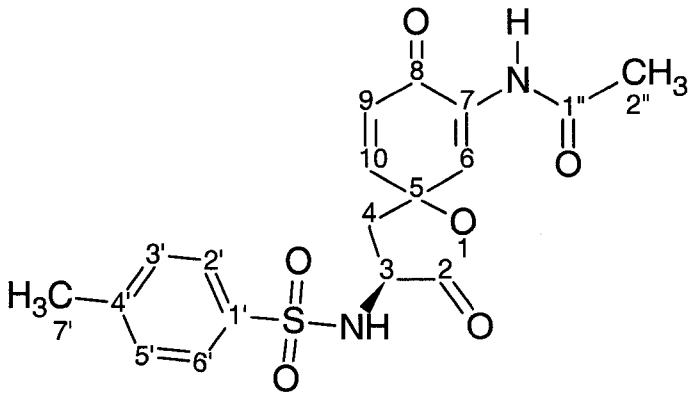


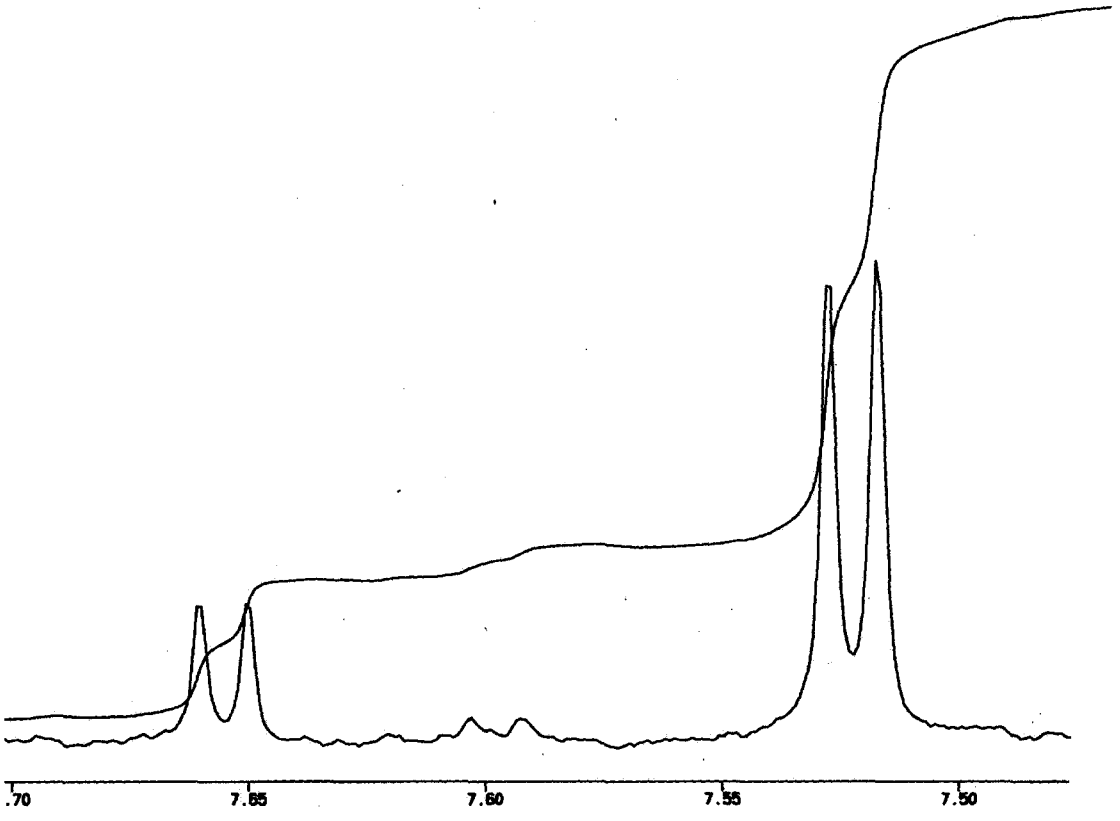
[1]H-NMR Spectrum of Crude Product (+)-**79/80**, Diastereomers



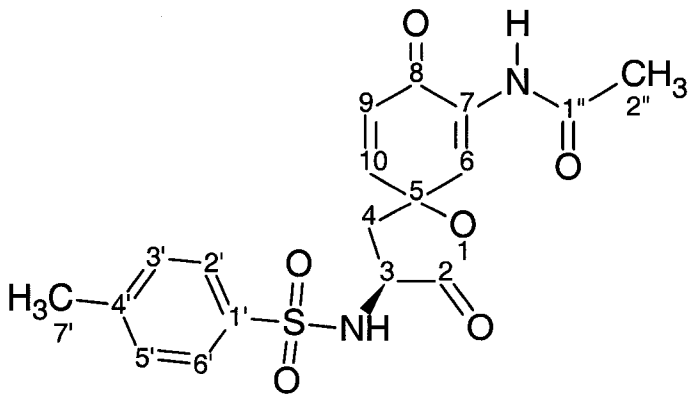


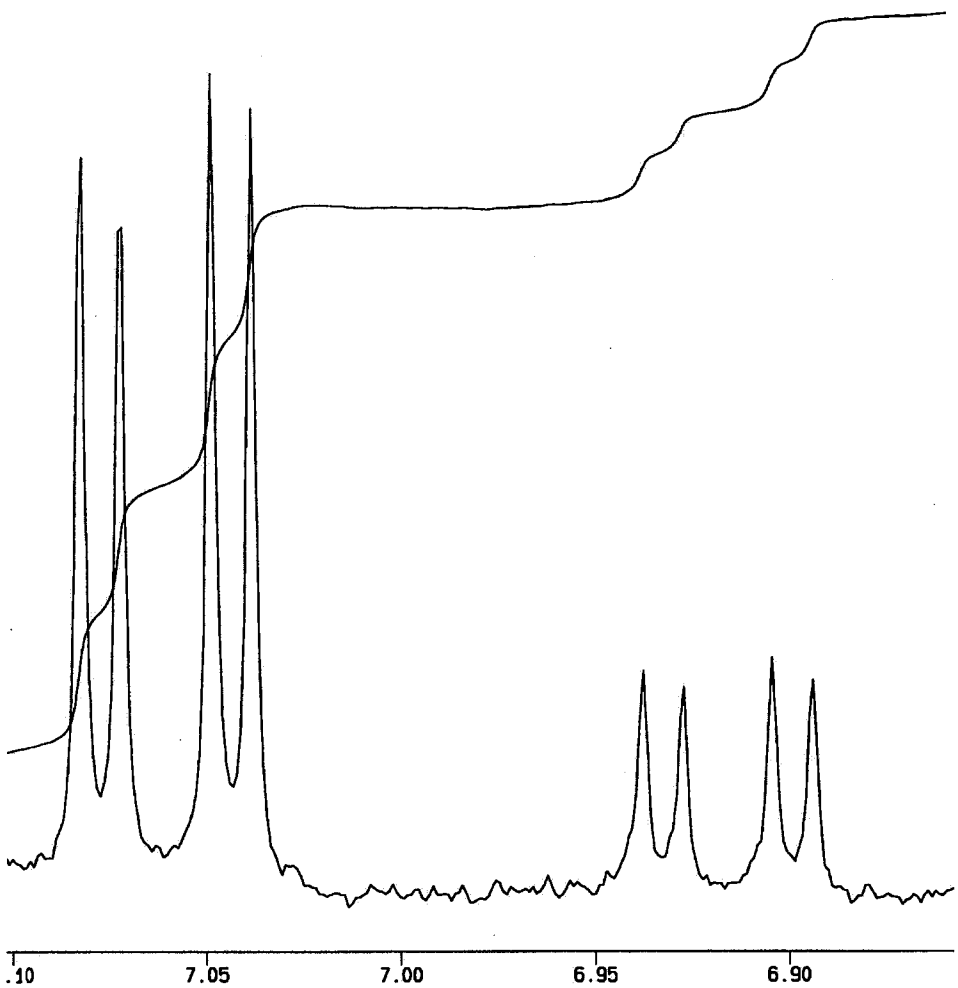
^{1}H-NMR Spectrum of Crude Product (+)-**79/80**, Diastereomers



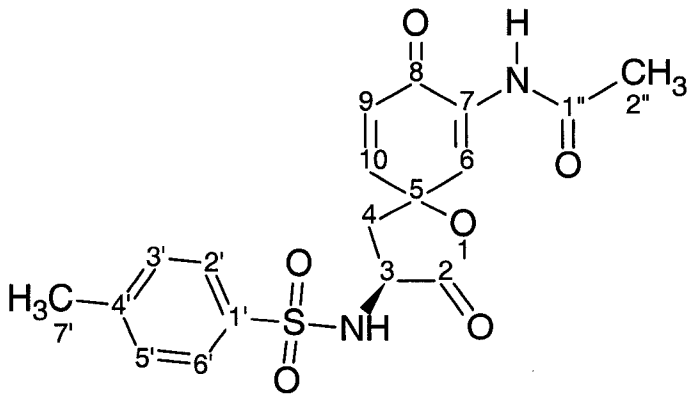


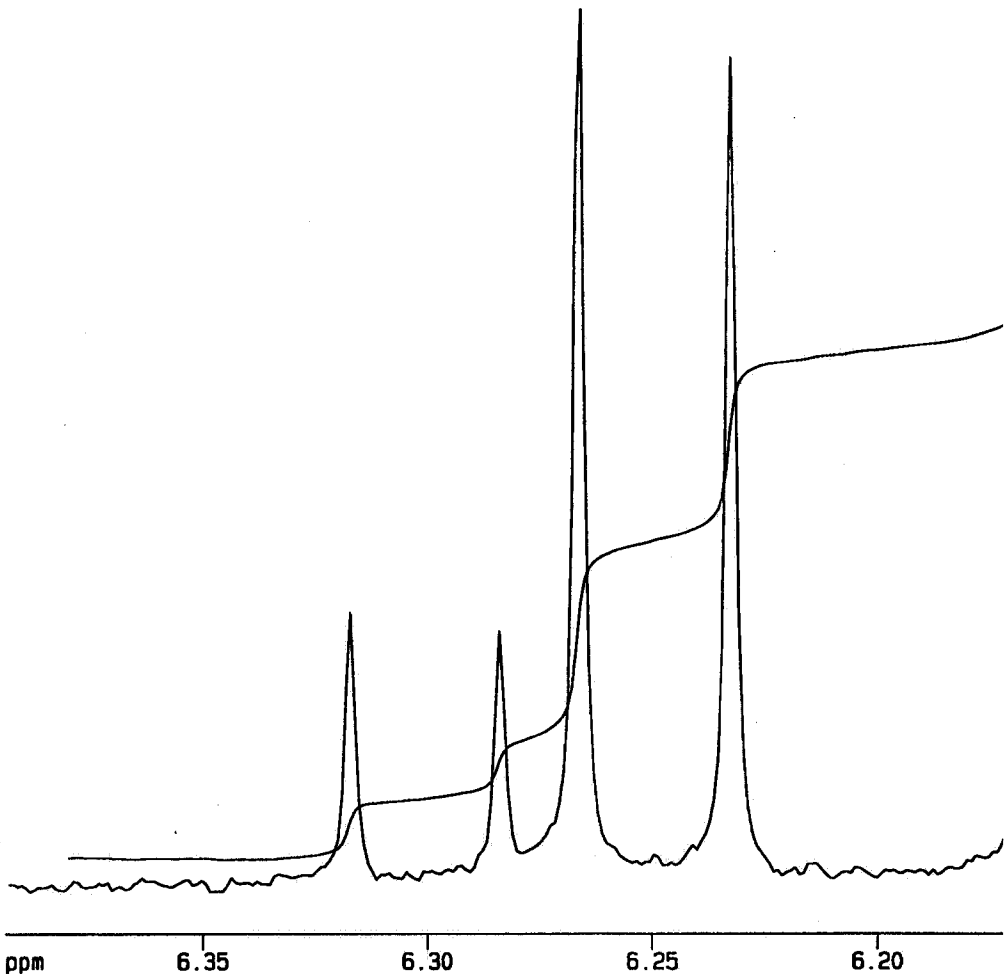
^{1}H-NMR Spectrum of Crude Product (+)-**79/80**, Diastereomers





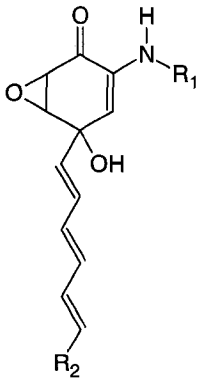
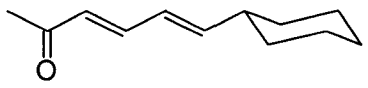
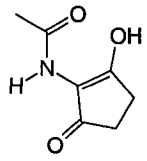
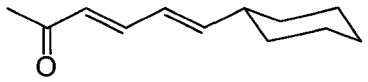
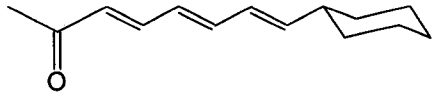
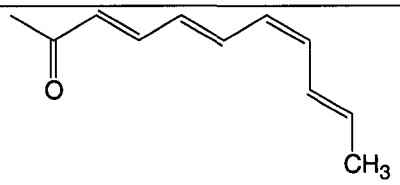
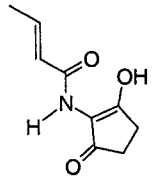
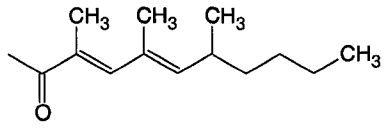
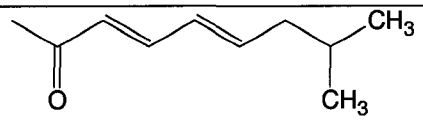
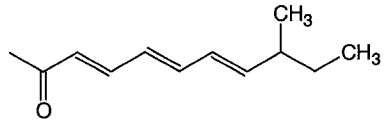
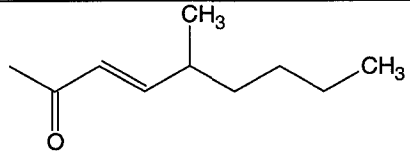
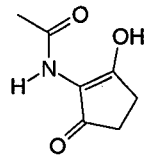
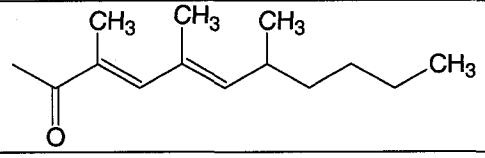
^{1}H-NMR Spectrum of Crude Product (+)-**79/80**, Diastereomers

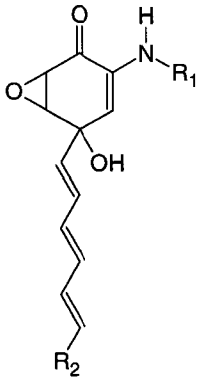
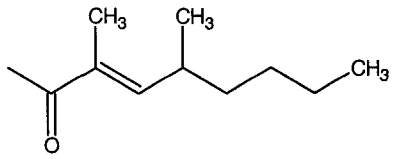
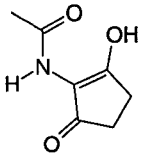
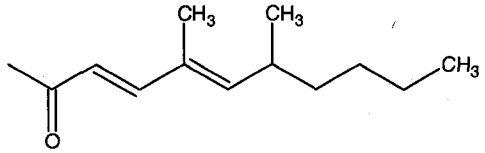
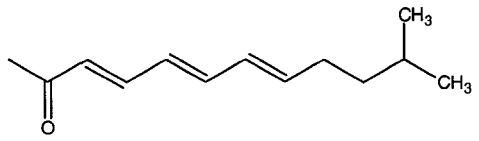
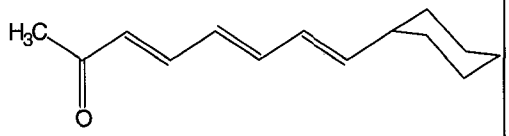
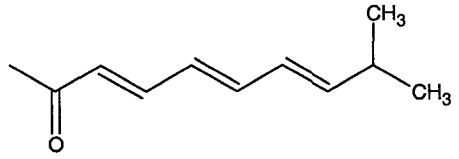
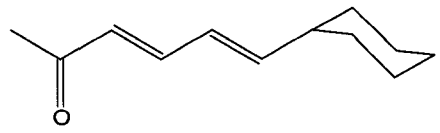
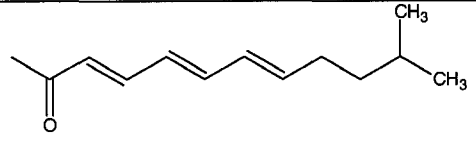




A Table of Manumycin Structures [7, 11]

Appendix 2

	Compound	R^1	R^2
Type I-a			
	Alisamycin		
	Ent-alisamycin		
	Asukamycin		
	Colabomycin A		
	Compound 1		CO_2H
	EI-1511-3		
	EI-1511-5		
	EI-1625-2		
	Manumycin A		

	Compound	R^1	R^2
Type I-a			
	Manumycin B		
	Manumycin C		
	Manumycin E		
	Manumycin F		
	Manumycin G		
	Nisamycin		CO_2H
	U-56, 407		

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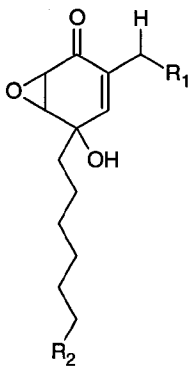
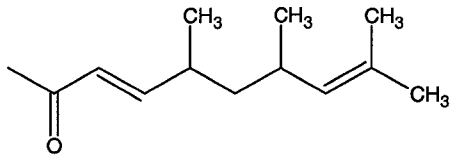
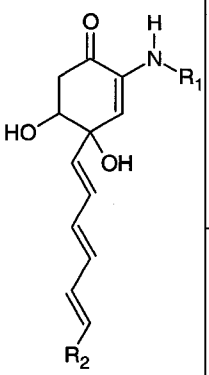
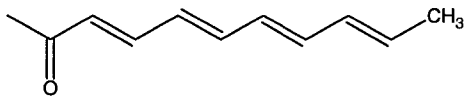
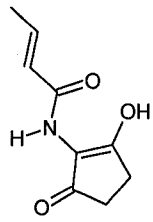
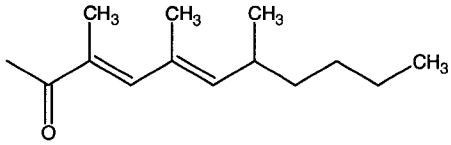
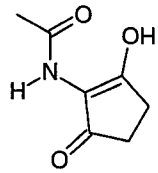
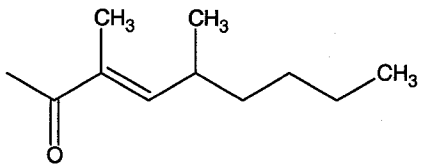
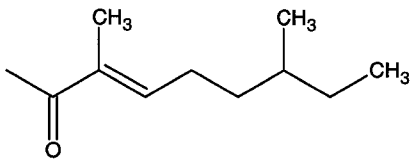
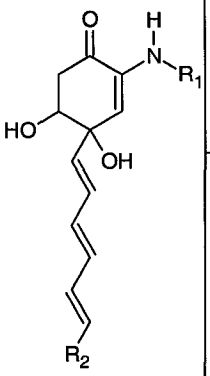
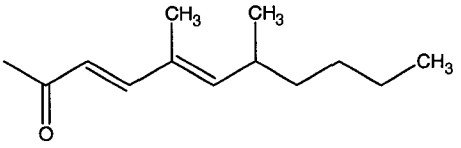
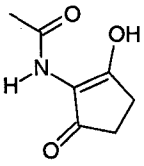
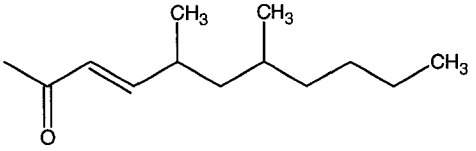
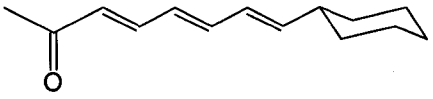
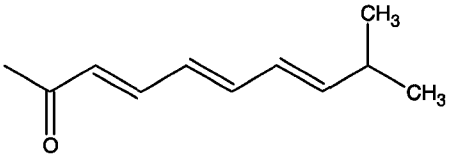
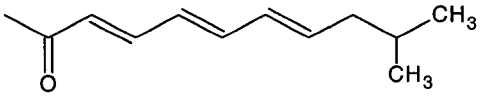
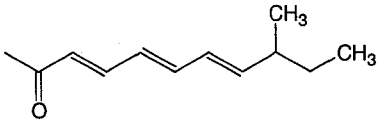
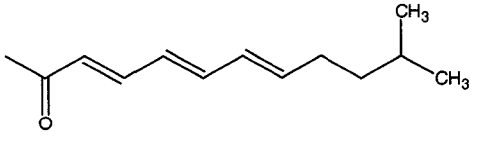
	U-62 162		CO_2H
Type II			
	Colabomycin D		
	Manumycin D		
	TMC-1A		
	TMC-1B		

	Compound	R^1	R^2
Type II			
	TMC-1C		
	TMC-1D		
	Asukamycin A-II		
	Asukamycin B-II		
	Asukamycin C-II		
	Asukamycin D-II		
	Asukamycin E-II		

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